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Antibiotic resistance and biofilm synthesis genes in airborne *Staphylococcus* in commercial aircraft cabins

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Abstract Passenger air transport is one of the main routes for the global spread of multidrug-resistant bacteria. This may be due to airborne pathogen transmission, which may occur within the commercial aircraft cabin. Because of this, we performed an investigation of aerial contamination by *Staphylococcus* species in 166 commercial aircraft and analyzed the presence of antibiotic resistance and biofilm synthesis genes in the collected isolates. Bacterial identification was performed by using species-specific primers and partial sequencing of 16S rRNA and *tuf* genes. The antibiotic resistance genes screened were: *mecA*, *mecC*, *blaZ*, *ermA*, *ermB*, *ermC,* and *vanA*. For biofilm synthesis, *ica* locus genes were screened. Fourteen species and four subspecies of *Staphylococcus* were detected in the analyzed samples. Except for *mecC* and *vanA*, all other genes were detected, including the *mecA* gene in *Staphylococcus aureus* and Coagulase-negative *Staphylococcus* isolates. Only *S. epidermidis* isolates were positive for biofilm formation. To date, this is the first study to report a significant diversity of airborne *Staphylococcus* and the presence of airborne methicillin-resistant

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Staphylococcus aureus (MRSA) in the cabin environment in commercial aircraft. Our results point to the importance of indoor air quality monitoring in the cabin environment as a preventive measure for the airborne spread of clinically significant pathogens.

Keywords Airborne bacteria · Aircraft · Antibiotic resistance · *Staphylococcus aureus* · Indoor air quality · Biofilm

1 Introduction

In indoor environments, air quality may be compromised by microorganisms responsible for various infections (Fujiyoshi et al., 2017). In these locations, aerial exposure to bacteria can lead to health problems such as disease, poisoning, and respiratory problems mediated by pro-inflammatory molecules (Holme et al., 2020). Inhalation of bacterial aerosols is related to tuberculosis, Q fever, Legionnaires' disease, pertussis, and pneumonia (de Rooij et al., 2016; Nardell, 2015; Warfel et al., 2012). Also, pathogens often involved in outbreaks of nosocomial infections such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and methicillin-resistant *Staphylococcus aureus* (MRSA), filamentous fungi and yeasts may have their transmission related to indoor air contamination (Fernstrom & Goldblatt, 2013; Hamzavi et al., 2019; Seiler et al., 2020).

Currently, indoor air quality issues have been the subject of extensive research because air pollution can cause serious health problems, including infectious diseases (Kulczyński et al., 2017). Analyses of bioaerosols' concentration in operating rooms of an educational hospital demonstrated potentially pathogenic microorganisms in indoor air, even after disinfection and sterilization procedures (Dehghani et al., 2018a). In this study, the authors highlighted factors related to the hospital building and the lack of proper management of post-surgical waste as responsible for bioaerosols' persistence even after mitigation procedures. In fact, many factors are described in the literature influencing the concentration of bioaerosols in indoor environments. In their study, Dehghani et al. (2018b) reported meteorological parameters such as temperature, relative humidity, and season influencing the concentration of fungal and bacterial bioaerosols in a wastewater treatment plant. In addition to these, air-conditioning systems, inadequate ventilation, building dampness, outdoor air, and humans' presence are other factors to be considered influencing bioaerosols' concentration (Dehghani et al., 2018a; Naddafi et al., 2019).

Studies addressing passengers and flight crew safety concerning exposure to airborne pathogens, especially in South American countries, are still scarce. Microorganisms can easily be transmitted within the aircraft cabin environment. Several factors are involved in this process, such as the presence of infected individuals, food handling, and improper maintenance of the climatization system (Leder & Newman, 2005). For bacteria, passenger air transport can be considered one of the main ways to contribute to its global spread, thus allowing these pathogens to overcome barriers and reach the most varied locations worldwide (Vila, 2015). In the context of air travel, inhalation of aerosolized bacteria may occur during (at the airport or aboard the aircraft) or after travel (upon arrival at the destination) (Petersen et al., 2017). In addition to the spread of disease, air travel also plays a significant role in the worldwide spread of antibiotic-resistant microorganisms. Schwartz and Morris (2018) reported the importance of air travel in the spread of antibiotic-resistant bacteria, highlighting the spread of critical multidrug-resistant bacteria. Extensively drug-resistant *Mycobacterium tuberculosis* (XDR-TB) within the cabin environment has also been reported in the literature (An der Heiden et al., 2017).

Of the few bacteria so far reported in commercial aircraft, MRSA is undoubtedly one that deserves much attention. This is because high mortality rates have been observed in infections caused by this pathogen. Until recently, its occurrence was restricted to the hospital environment only (Healthcare-associated methicillin-resistant *Staphylococcus aureus* HA-MRSA) (Lakhundi & Zhang, 2018). However, in recent years, several cases have been associated with community isolates of MRSA (community-associated methicillin-resistant *Staphylococcus aureus* CA-MRSA), involving mainly its transmission from environmental sources (Kong et al., 2016). In this context, air travel plays an essential role in CA-MRSA epidemiology as it can also contribute to its widespread environmental dissemination.

MRSA has been found in commercial aircraft contaminating various surfaces within the cabin

environment (Zhao et al., 2019). However, its occurrence in indoor air has not yet been reported. In addition to MRSA, members of the coagulase-negative *Staphylococcus* (CoNS) group also stand out as significant opportunistic pathogens. *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Staphylococcus haemolyticus*, *Staphylococcus capitis*, *Staphylococcus saprophyticus*, and *Staphylococcus lugdunensis* are the main causes of human infections, especially those associated with the use of hospital devices (Becker et al., 2014).

Besides antibiotic resistance, *Staphylococcus* sp. also stand out as important biofilm formers. This structure comprises communities of microorganisms encased in a polysaccharide matrix adhered to a surface (Gün & Ekİncİ, 2009). The main polysaccharide present in the *Staphylococcus* biofilms is polysaccharide intercellular adhesin (PIA), and its synthesis is mediated by *ica*ADBC locus (Arciola et al., 2015). The *ica*A, *ica*D, *ica*B, *ica*C genes, and the *ica*R promoter are part of the *ica* locus's composition. The coexpression of *ica*A and *ica*D is the main mechanisms involved in forming the biofilm structure (Omidi et al., 2020).

One of the most significant impacts of biofilm formation is related to bacteria's ability to adhere to medical devices' surfaces, which contributes to the occurrence of infections related to their use. Additionally, biofilm-associated infections provide bacterial cells with the ability to resist immune responses from the host organism and resistance to the action of antibiotics, further aggravating the problem (Høiby et al., 2010). Some of the mechanisms related to the increase in bacterial resistance to antibiotics in biofilms involve: favoring horizontal gene transfer, low penetration of the drug in the biofilm matrix, overexpression of enzymes that degrade antibiotics, imprisonment of the molecule in the biofilm structure, and its destruction by specific enzymes and modification of drug target molecules (Gebreyohannes et al., 2019).

Considering the scarcity of studies on the microbiological contamination of indoor air in aircraft, it is of great importance to gather evidence that can guide discussions about Indoor Air Quality (IAQ) in this specific environment. Thus, this study aimed to evaluate aerial contamination by *Staphylococcus* species in commercial aircraft, checking for the presence of antibiotic resistance genes and biofilm synthesis in the collected isolates. Our results provide a preliminary overview that could guide the development of standards and regulations to improve indoor air quality and passenger and crew safety in commercial aircraft.

2 Methods and materials

2.1 Air collections

Was evaluated the microbiological air quality in aircraft that landed at the Zumbi dos Palmares International Airport, located in Maceió city, Alagoas/Brazil. Were selected commercial aircraft from five airlines operating on national and international routes to investigate bacterial bioaerosols. The monitoring was carried out from January 2018 to December 2018. The national flights originated in the cities of Brasilia, São Paulo, Rio de Janeiro, Recife, and Belo Horizonte. The international route was Buenos Aires-Maceió.

A monthly monitoring plan was developed and followed throughout the study. This plan consisted of going to the airport to carry out air sampling on the aircraft and was carried out as follows: January to April, six monitoring sessions per month; May and June, four monitoring sessions per month; July, three monitoring sessions; August to December, two monitoring each month (Online Resource 1). At the end of the study, 166 commercial aircraft were evaluated for aerial contamination by *Staphylococcus* sp. during the 45 days of monitoring. Since no legislation in Brazil regulates indoor air quality monitoring in commercial aircraft, we only received authorization for collections to be carried out after the aircraft landed and passengers disembarked. Access to the aircraft was only possible with the responsible commander's permission and the support of inspection agents from Brazil's National Health Surveillance Agency. The collection of samples and the evaluation of indoor air quality followed the recommendations of the Brazilian legislation on air quality in artificially conditioned environments (ANVISA 2003). It was used as a parameter due to the absence of national and international air quality legislation on commercial aircraft.

2.2 Collection procedures and bacterial aerosol analysis

Samples were obtained using a SAS Super ISO 100 portable bioaerosol collector (VWR Collection), positioned approximately 1.5 m above the cockpit and passenger cabin floor. Indoor air samples were collected for three minutes at an airflow rate of 100 L/min. The bioaerosol sampler was calibrated and sanitized before utilizing it in the air collections and cleaned with 95% isopropyl alcohol at the end of each sampling to avoid cross-contamination. The aircraft chosen to assess the microbiological quality of indoor air were: Boeing 737–800 from Airline 1, Airbus A320 and A321 from Airline 2 and Airline 4, and Embraer ATR 72–600 and E195 from Airline 3. These aircraft make flights to Maceió. Journey time for domestic routes was no longer than three hours, while for the international route the duration was 5 h and 20 min. All aircraft evaluated were traveling at or near maximum passenger capacity. According to information provided by the airlines, the passenger capacity reported for the different equipment evaluated was: A320 and A321, 185 and 220 passengers, respectively, Boeing 737–800, 199 passengers; ATR 72–600, 72 passengers; E195, 124 passengers. In addition to the aircraft's collections, outdoor air sampling in the airport courtyard was also performed to verify its possible influence on the concentration of bioaerosols inside the analyzed cabin.

For bacterial growth, disposable petri dishes containing blood agar medium were incubated at 37 °C for 48 h. After growth, colonies with *Staphylococcus* morphotypes (generally smooth, convex, continuous border and white-porcelain or gray colored) were selected and purified on plates containing BHI (Brain Heart Infusion) agar medium. Pure cultures grown in 1250-µL BHI medium at 37 °C for 48 h were used for DNA extraction and glycerol storage.

2.3 Quality control

All procedures related to indoor air analysis followed the Brazilian legislation on air quality in artificially conditioned environments (ANVISA 2003). Culture media were prepared based on the manufacturer's instruction, and the sterility of media was checked by incubating 5% of the plates at 35–37 °C overnight and observing bacterial growth. Culture media that showed growth were discarded. Quality control procedures were in according with Chegini et al. (2020).

2.4 Molecular identification of bacterial aerosols

The DNA extraction of the collected samples followed the protocol of Dashti et al. (2009), with modification carried out in the last stage of the protocol, adding chloroform/isoamyl alcohol (24:1) to obtain a purer DNA. In summary, one thousand microliters of pure culture was centrifuged at 15,000 g for 5 min. Then, the supernatant was discarded, and the pellet was resuspended in 1 mL of sterile Milli-Q H_2O, with further centrifugation at 15,000 g for 5 min. After centrifugation, the pellet was resuspended in 200 µL TE Buffer (10 mM/1 mM Tris–EDTA, pH 8.0) and boiled at 99 °C for 15 min. After heating, the tubes were frozen in a − 20 °C freezer for 15 min, and after this time, the samples were kept at room temperature until thawing, when 500 µL chloroform/isoamyl alcohol (24: 1) was added. After further centrifugation at 15,000 g for 10 min, 150 µL of the DNA-containing supernatant was transferred to a new tube and stored in a − 20 °C freezer until analysis.

For molecular identification, PCRs were performed using primers already described in the literature. Initially, specific primers were used to amplify the 16S rRNA gene fragment, as it is a widely used approach in bacterial identification studies (Barbosa et al., 2018). In addition to this gene, primers that amplified a fragment of the *Staphylococcus tuf* gene, an alternative marker with better discriminating power for this genus species, were also used (Bergeron et al., 2011). Specific primers to the major disease-associated species in humans were also used to identify isolates (Hirotaki et al., 2011). All conditions for PCR followed the procedures described in the literature, except for annealing temperatures which in some cases were modified. Primer sequences, amplicon size, and annealing temperatures are shown in Table 1.

PCRs were prepared in 25 µL final volume in a 0.2-mL microcentrifuge tubes containing: 2.5 µL 10X PCR buffer (100 mM Tris–HCl pH 8.5, 500 mM KCl), 2.5 µL dNTPs (20 µM), 1.0 µL (0.4 µM) of each oligonucleotide, 1.5 µL MgCl2 (50 mM), 0.25 µL Taq DNA Polymerase (5 U/µL), 1 µL genomic DNA, and 15.25 µL of ultrapure water. All reactions occurred in a Peltier Thermal Cycler MJ25 + (MJ Research/Bio-Rad). After amplifying the 16S rRNA

Table 1 Primers used for molecular identification of airborne *Staphylococcus* species

Primers	Gene	Sequence (5'–3')	Annealing	Amplicon (pb)
BG1	16S rRNA	AGACTCCTACGGGAGGCAGC	60 °C	~ 500
BG2		GGACTACCAGGGTATCTAATCC		
stat1	*Staphylococcus* – *tuf* gene	TTATCACGTAACGTTGGTG	53 °C	660
stat2		CATTTCWGTACCTTCTGG		
Staphylococcus hominis	*nuc* gene	TACAGGGCCATTTAAAGACG	52 °C	177
hom-F		GTTTCTGGTGTATCAACACC		
hom-R				
Staphylococcus epidermidis	*nuc* gene	TTGTAAACCATTCTGGACCG	52 °C	251
epi-F		ATGCGTGAGATACTTCTTCG		
epi-R				
S. aureus	*nuc* gene	TCGCTTGCTATGATTGTGG	52 °C	359
aur-F		GCCAATGTTCTACCATAGC		
aur-R				
Staphylococcus haemolyticus	*nuc* gene	TAGTGGTAGGCGTATTAGCC	52 °C	434
hae-F		ACGATATTTGCCATTCGGTG		
hae-R				
Staphylococcus capitis	*nuc* gene	ACTACGCCTATGATTATTGC	51 °C	525
cap-F		GAYGCTTCTTTACCATAGGG		
cap-R				
Staphylococcus lugdunensis	*nuc* gene	TCCAATGATGGTAACGAGGC	58 °C	695
lug-F		TTTTGCGCCTCGTTTTGTGC		
lug-R				
Staphylococcus saprophyticus	*nuc* gene	TTTTGGATGCGATAGATTGG	51 °C	843
sap-F		TCTTCAGACTTTTCAAAGGC		
sap-R				
Staphylococcus warneri	*nuc* gene	CGTTTGTAGCAAAACAGGGC	53 °C	999
war-F		GCAACGAGTAACCTTGCCAC		
war-R				

and *tuf* gene products, a 5-µL aliquot of PCR products was applied to 1.2% agarose gel and electrophoresed in TBE buffer (Tris-Boric Acid, 0.5 M EDTA pH 8.0) using 85 V/cm for one hour and 20 min. After electrophoresis, the gel was stained in ethidium bromide for two minutes and photographed using a camera (Sony Cyber-shot® DSC-W510) in an ultra-violet light transilluminator (312 nm wavelength). PCR products were sent for sequencing at Macrogen Inc. (Seoul, South Korea).

The sequences obtained were evaluated to verify the reliability of each nucleotide based on PHRED values > 30, followed by consensus assembly using the Staden Package software (Staden et al., 2003). Local alignments were performed using the Basic Local Alignment Search Tool (BLAST) for preliminary identification of the sequenced products (Altschul et al., 1990). For 16S rRNA gene, species-level identification occurred when a sequence similarity value above 99% was observed, while for the *tuf* gene the homology values between sequences were $\geq 98\%$.

Paired comparisons between the sequences obtained and bacterial sequences already identified and available on GenBank (https://www.ncbi.nlm.nih.gov/genbank/) were performed. For this analysis, we used the Species Demarcation Tool v. 1.0 software to estimate the identity percentage between nucleotide

sequences (Muhire et al., 2013). Sequences corresponding to the *Staphylococcus tuf* gene were aligned using the CLUSTAL W algorithm implemented in MEGA 6 software (Molecular Evolutionary Genetics Analysis) (Tamura et al., 2011). Phylogenetic analyses were performed by the Neighbor-Joining (NJ) method, applying the Kimura-2-parameter model (K2P) using the SeaView 4 software (Gouy et al., 2009). The reliability of the generated tree was obtained using the bootstrap support statistical test with a value of 1000 random replicates. *Macrococcus caseolyticus* was used as outgroup.

2.5 Antibiotic resistance and biofilm synthesis gene screening

Given the relevance of antibiotic resistance in *Staphylococcus* sp., genes related to lower susceptibility to some drugs used to treat diseases caused by these bacteria were screened. The genes investigated were *mecA* and *mecC* (methicillin/oxacillin resistance), *blaZ* (penicillin resistance), *ermA*, *ermB* and *ermC* (cross-resistance to macrolides, lincosamides, and streptogramin B), and *vanA* (vancomycin resistance). The analysis was performed by using primers already described in the literature (Table 2).

Apart from antibiotic resistance, *Staphylococcus* species are also known for the formation of biofilms, which are important virulence factors for infection success. Thus, all five individual genes of the *ica* locus (*ica*ADBC and promoter region *ica*R) responsible for the biofilm synthesis in *Staphylococcus* sp. were scanned. Specific primers were used to detect each gene (Table 2), as described by Arciola et al. (2005). The parameters for amplification of the fragments of interest were those already described in the literature, with modification of the annealing temperature in the reaction to detect the *blaZ* gene, which was 53 °C. The final reaction volume and reagent concentration were the same used for the identification reactions.

2.6 Data processing and statistical analysis

We performed a comparison of the frequencies found with frequencies expected at random using the chi-square test for a simple sample with residual analysis in each category. Also, a comparison of the distributions of the number of isolates detected in indoor x outdoor air was made using the Mann–Whitney test. Was adopted alpha equal to 5% in all analyses and utilized the statistical software Stata v 13.0 (StataCorp, College Station, TX).

3 Results

3.1 Indoor and outdoor airborne Staphylococcus

Of the total days on which monitoring was carried out, in 11/45 (24.4%) days, bacterial isolates were detected only in indoor air samples, while in 1/45 (2.2%) days, they were detected only in outdoor air samples. In 7/45 (15.6%) days, we obtained isolated in both indoor and outdoor air. Airborne *Staphylococcus* were not detected in 26/45 (57.8%) days.

Considering only the 19 days in which there was some detection, it is clear that the frequency of detection combinations (yes-indoor + non-outdoor; non-indoor + yes-outdoor; and yes-indoor + yes-outdoor) was statistically different than would be expected by chance, according to the one-sample Chi-square test ($\chi^2 = 8.0$, df = 2, $P = 0.01$). When analyzing the residuals, the combination "yes-indoor + no-outdoor" presented the largest residual (4.7), while "no-indoor + yes-outdoor" presented the lowest residual (− 5.3). The "yes-indoor + yes-outdoor" combination showed an almost neutral residue (0.7). We observed that the "Yes-indoor + no-outdoor" combination showed a way higher frequency than what would be expected by chance, as observed by the residuals, and the "no-indoor + yes-outdoor" combination showed a way lower frequency than the expected. These results indicate that the presence of indoor bioaerosols was not dependent on the presence of outdoor bioaerosols.

The average of isolates detected in indoor air was 9.2 isolates, with a standard deviation of 10.6, while in outdoor air, the average was 2.6 isolates with a standard deviation of 2.3. The medians were 6.5 and 1, respectively. When performing a nonparametric Mann–Whitney comparison for these distributions, we found that the average rank of the distribution of the number of indoor isolates (16.22) is significantly higher than the average rank of the distribution of the number of outdoor isolates (7.38; $P < 0.01$). These results show statistical significance, indicating that the number of isolates indoors is significantly higher than the number of isolates outdoor.

Table 2 List of primers used for screening antibiotic resistance and biofilm synthesis genes in airborne *Staphylococcus* species

Primers	Gene	Sequence (5'–3')	Amplicon (pb)	Reference
MRS1	*mecA*	TAGAAATGACTGAACGTCCG	154	Pereira et al. (2010)
MRS2		TTGCGATCAATGTTACCGTAG		
mecC-F	*mecC*	GAAAAAAAGGCTTAGAACGCCTC	138	Pajić et al. (2014)
mecC-R		GAAGATCTTTTCCGTTTTCAGC		
blaZ-1	*blaZ*	ACTTCAACACCTGCTGCTTTC	173	Duran et al. (2012)
blaZ-2		TGACCACTTTTATCAGCAACC		
ermA F	*ermA*	TATCTTATCGTTGAGAAGGGATT	139	Kareem et al. (2015)
ermA R		CTACACTTGGCTTAGGATGAAA		
ermB F	*ermB*	CTATCTGATTGTTGAAGAAGGATT	142	
ermB R		GTTTACTCTTGGTTTAGGATGAAA		
ermC F	*ermC*	CTTGTTGATCACGATAATTTCC	190	
ermC R		ATCTTTTAGCAAACCCGTATTC		
vanA F	*vanA*	GGCAAGTCAGGTGAAGATG	713	Azimian et al. (2012)
vanA R		ATCAAGCGGTCAATCAGTTC		
icaA F	*icaA*	ACAGTCGCTACGAAAAGAA	103	Arciola et al. (2005)
icaA R		GGAAATGCCATAATGACAAC		
icaB F	*icaB*	CTGATCAAGAATTTAAATCACAAA	302	
icaB R		AAAGTCCCATAAGCCTGTTT		
icaC F	*icaC*	TAACTTTAGGCGCATATGTTTT	400	
icaC R		TTCCAGTTAGGCTGGTATTG		
icaD F	*icaD*	ATGGTCAAGCCCAGACAGAG	198	
icaD R		CGTGTTTTCAACATTTAATGCAA		
icaR 1	*icaR*	TAATCCCGAATTTTTGTGAA	469	
icaR 2		AACGCAATAACCTTATTTTCC		

3.2 Identification of Staphylococcus species

After 45 days of monitoring, 166 air samplings in commercial aircraft and 45 outdoor air samplings were performed, with 187 positive isolates for the genus *Staphylococcus*. Out of the total isolates, 166 (88.8%) were obtained from indoor air collection, while 21 (11.2%) were from the outdoor air. Fourteen species and four subspecies of *Staphylococcus* were identified (Table 3). Phylogenetic analyses and paired comparisons of partial *tuf* gene sequences confirmed the identification (Fig. 1). Some sequences did not have enough quality to permit specific identification at species level and are described at the genus level.

Clinically significant species such as *S. aureus*, *S. epidermidis*¸ *S. saprophyticus,* and *S. haemolyticus* were present inside the evaluated aircrafts. Species *S. epidermidis*, *S. hominis*, *S. cohnii*, *S. aureus*, and *S. warneri* were the most frequent, while *S. carnosus*, *S. gallinarum*, and *S. sciuri* were found less frequently, with only one isolate for each species being identified. (Fig. 2). In outdoor air samples, *S. aureus*, *S. haemolyticus*, *S. saprophyticus*, *S. capitis*, *S. arlettae, S. hominis*, and *S. cohnni* were found.*n*

3.3 Antibiotic resistance gene detection

The *mecA*, *blaZ*, *ermA*, *ermB*, and *ermC* genes were detected in airborne *Staphylococcus*. No isolates were positive for the detection of *mecC* and *vanA* genes. Out of the isolates obtained inside the aircraft, 126/166 (75.9%) were positive for carrying the screened resistance genes (Fig. 3 and 5). Some isolates carried more than one gene, while others displayed only isolated genes (Table 4). Regarding each gene's detection frequency in the positive samples, the *blaZ*

Table 3 Airborne *Staphylococcus* species identified based on partial sequencing of 16S rDNA and *tuf* genes

Sample code	Molecular identification	Gene	Similarity (%)	Reference (GenBank)
AB110	*Staphylococcus arlettae*	16S rDNA	100	MK095131
AB115	*Staphylococcus hominis subsp. novobiosepticus*	16S rDNA	99	NR041323
AB345	*Staphylococcus sciuri*	16S rDNA	100	Z26901
AB348	*Staphylococcus gallinarum*	16S rDNA	100	MK097364
AB372	*Staphylococcus saprophyticus*	16S rDNA	100	MK026832
AB397	*Staphylococcus carnosus*	16S rDNA	100	Z26891
AB419	*Staphylococcus haemolyticus*	16S rDNA	100	KR779801
AB155	*Staphylococcus hominis*	*tuf*	100	AF298802
AB156	*Staphylococcus cohnii subsp. ureolyticus*	*tuf*	99	AF298799
AB158	*Staphylococcus capitis*	*tuf*	100	AF298798
AB161	*Staphylococcus epidermidis*	*tuf*	100	AF298800
AB164	*Staphylococcus aureus*	*tuf*	99	CP032051
AB167	*Staphylococcus saprophyticus subsp. bovis*	*tuf*	100	EU652815
AB169	*Staphylococcus kloosii*	*tuf*	99	EU652813
AB221	*Staphylococcus cohnii*	*tuf*	99	CP027422
AB234	*Staphylococcus pasteuri*	*tuf*	100	CP017463
AB342	*Staphylococcus aprophyticus subsp. saprophyticus*	*tuf*	99	LR134089
AB414	*Staphylococcus warneri*	*tuf*	99	AF298806

AB Airborne bacteria

gene was the most frequent among the isolates, followed by *ermC*, *ermA*, and *mecA* (Fig. 4). We found the *mecA* gene in *S. aureus*, *S. warneri*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. pasteuri,* and *S. sciuri*. Antibiotic resistance genes were also detected in isolates obtained from outdoor air, except for *vanA* and *mecC* genes. In these samples, detection of the *mecA* gene was observed in only one *S. haemolyticus* isolate.

3.4 Biofilm synthesis gene detection

All genetic elements that make up the *ica* locus (ADBC) and the *ica*R promoter region were detected in airborne *Staphylococcus* obtained from commercial aircraft (Fig. 5). Out of the total isolates, 67/166 (40.4%) were positive for *ica* locus gene detection (Table 4). The *ica*R gene was found most frequently among the isolates, followed by *ica*D and *ica*A genes, respectively (Fig. 6). In *Staphylococcus* species, biofilm formation is mediated mainly by the joint expression of *ica*A and *ica*D genes. Analyzing the joint detection of *ica*AD in our isolates, only 16/166 (9.6%) were positive, occurring only in *S. epidermidis*. Regarding isolates from outdoor air, except for *ica*C, all other genes were detected, and no joint detection of *ica*AD was observed in the samples analyzed.

4 Discussion

The aircraft cabin currently used in world civil aviation has been considered an environment with the low introduction of microorganisms harmful to human health, mainly because they are equipped with air filters with high-efficiency particulate arrestance (HEPA filters) (Mangili et al., 2015). However, there is currently no legislation that seeks to supervise the efficiency, periodicity of operation and proper maintenance of those filters (Pavia, 2007). Thus, monitoring the air quality offered to passengers and crew is a valuable source of information for better understanding disease transmission dynamics in this environment, leading to a safer flight transport condition.

In this study, we report the indoor air contamination in commercial aircraft by *Staphylococcus* sp, including the presence of potentially pathogenic species such as *S. aureus*, *S. epidermidis*, *S. saprophyticus*, and *S.*

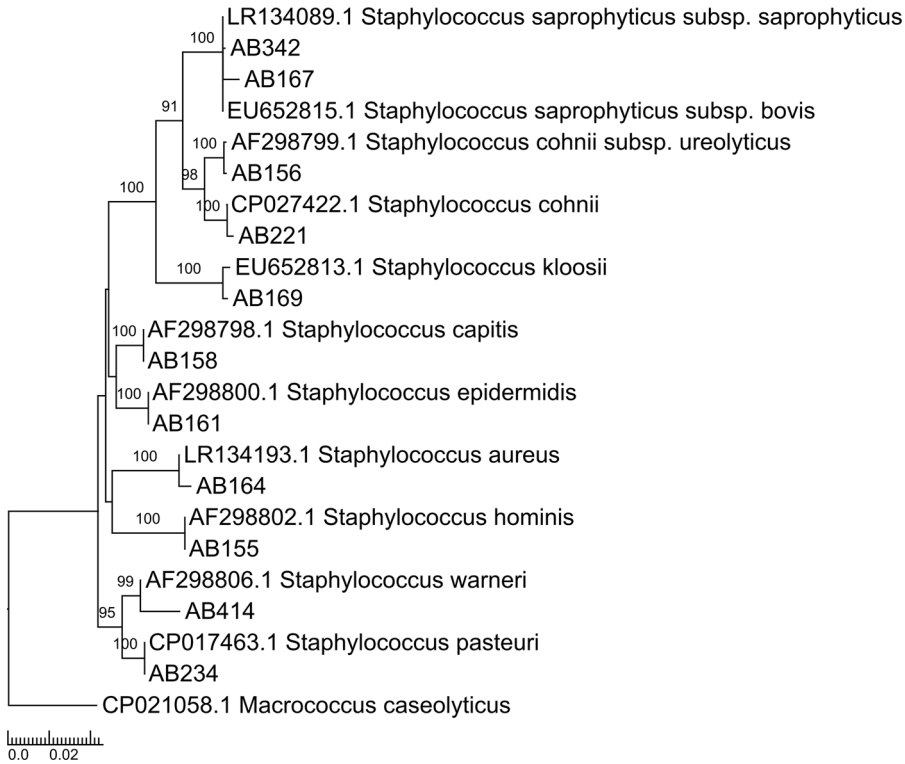

Fig. 1 Neighbor joining phylogenetic tree generated by SeaView 4 software, built based on multiple alignment of partial sequences of the *Staphylococcus tuf* gene. The nucleotide substitution model used was Kimura-2-parameter (K2P). Node numbers indicate percentage bootstrap values (1000 bootstraps). *Macrococcus caseolyticus* was used as outgroup

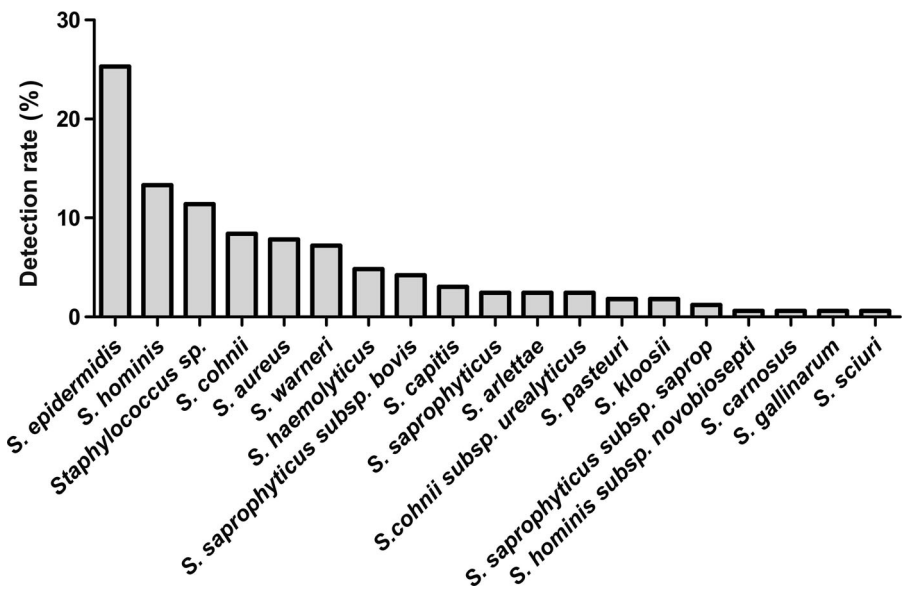

Fig. 2 Detection rate of *Staphylococcus* species on air samples from commercial aircraft (n = 166)

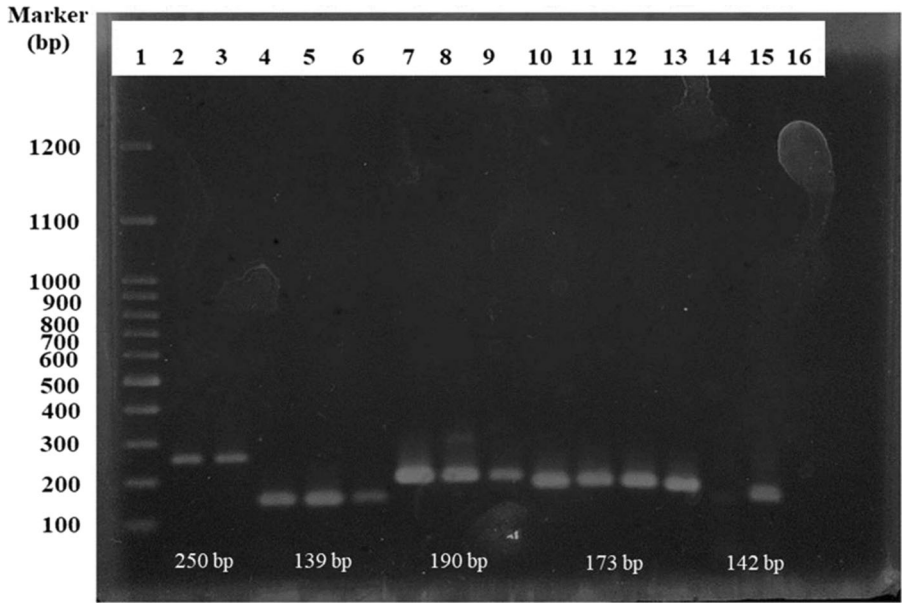

Fig. 3 Detection of antibiotic resistance genes in airborne *Staphylococcus*. Electrophoresis on a 1.2% agarose gel. Lanes: 4–6, *ermA* gene; 7–9, *ermC* gene; 10–13, *blaZ* gene; 15, *ermB* gene

haemolyticus. These results provide additional evidence that indoor air is an essential carrier of clinically meaningful bacteria, contributing to outbreaks and spread diseases at national and international levels. *Staphylococcus* sp. are often present in indoor air quality monitoring and have been reported in places such as hospitals, subway stations, homes, and commercial buildings (Asif et al., 2018; Mirhoseini et al., 2016). However, studies describing indoor air contamination in aircraft by these microorganisms are still scarce.

Although investigations on air quality show a great diversity of these bacteria in various environments, few species have been reported in the aircraft cabin. In contrast to our results, Dechow et al. (1997) did not find a diversity of *Staphylococcus* sp. in air samples collected inside the cabin environment during the flight, reporting only the detection of *S. epidermidis*. Likewise, this species was the most frequent in our study. Osman et al. (2008) also note a great diversity of airborne *Staphylococcus* within the cabin environment on short- and long-haul flights. Although we have detected a more significant number of species, the authors report *S. hominis* and *S. epidermidis* as the most frequent species in this same study. Unlike them, *S. epidermidis* and *S. hominis* were most frequent in our samples. The detection of these bacteria and other species such as *S. cohnii*, *S. aureus*, and *S. warneri* in greater frequency in the evaluated aircraft may be explained by the fact that all these species are part of the human microbiome found on the skin and mucous membranes. Thus, passenger's and crew's behavior inside the aircraft may have contributed to these bacteria's aerosolization in the cabin environment. Corroborating this, Han et al. (2014) found that some behaviors such as speech, cough, and displacement within the aircraft tend to increase infection risk through exposure to airborne pathogens. Furthermore, the incredible versatility of *Staphylococcus* sp. allows them to survive under different conditions on surfaces and dust particles (White et al., 2020), which could also explain their presence in the evaluated aircraft.

Staphylococcus carnosus, *Staphylococcus gallinarum*, and *Staphylococcus sciuri* were the least frequent air samples in our monitoring. We obtained only one isolate for each species. These species appear to occur at a low frequency as indoor air contaminant. Few studies report their detection in various indoor air environments and no detection in the aircraft cabin environment. Similar to our results, *S. gallinarum* was detected in indoor air samples from university-built environments (Yassin & Almouqatea, 2010), and *S. carnosus* and *S. sciuri* were reported as indoor air contaminants in the office buildings. Compared to

Table 4 Antibiotic resistance and biofilm synthesis genes in airborne *Staphylococcus* of commercial aircraft and outdoor air samples

Isolate code	Species	Antibiotic resistance gene	Biofilm synthesis gene
AB03	*Staphylococcus epidermidis*	*blaZ*	
AB04	*Staphylococcus epidermidis*	*blaZ*	
AB10	*Staphylococcus epidermidis*	*blaZ*	
AB11	*Staphylococcus epidermidis*	*blaZ*	
AB13	*Staphylococcus epidermidis*	*blaZ, ermC*	*icaD, icaR*
AB15	*Staphylococcus epidermidis*	*blaZ, ermC*	*icaD, icaR*
AB16	*Staphylococcus epidermidis*	*blaZ, ermC,*	*icaD, icaR*
AB31	*Staphylococcus warneri*	*blaZ*	
AB32	*Staphylococcus hominis*	*blaZ, icaC,*	*icaD, icaR*
AB34	*Staphylococcus epidermidis*	*blaZ*	*icaA, icaB, icaC, icaD, icaR*
AB36	*Staphylococcus epidermidis*	*mecA, blaZ, ermC*	*icaA, icaD, icaR*
AB41	*Staphylococcus aureus*	*mecA, blaZ, ermC*	*icaB, icaD, icaR*
AB44	*Staphylococcus epidermidis*	*blaZ, ermA*	*icaA, icaB, icaC, icaD, icaR*
AB54	*Staphylococcus epidermidis*	*blaZ,*	*icaA, icaB, icaC, icaD, icaR*
AB57	*Staphylococcus haemolyticus*	*mecA, blaZ*	*icaR*
AB58	*Staphylococcus warneri*	*mecA*	
AB59	*Staphylococcus haemolyticus*	*blaZ*	*icaR*
AB80	*Staphylococcus hominis*	*mecA, blaZ*	*icaD*
AB82	*Staphylococcus saprophyticus*	*blaZ,*	*icaC, icaD*
AB83	*Staphylococcus cohnii subsp. urealyticus*	*blaZ*	*icaD*
AB96[a]	*Staphylococcus* sp.	*blaZ*	*icaD, icaR*
AB110	*Staphylococcus arlettae*	*blaZ, ermB,*	*icaB, icaR*
AB115	*Staphylococcus hominis subsp. novobiosepticus*	*blaZ, ermC*	*icaD, icaR*
AB116	*Staphylococcus warneri*	*blaZ, ermA*	*icaA*
AB117	*Staphylococcus epidermidis*	*blaZ, icaR*	
AB123	*Staphylococcus warneri*	*blaZ,*	
AB124	*Staphylococcus haemolyticus*	*blaZ,*	
AB126	*Staphylococcus warneri*	*blaZ, ermA*	
AB129	*Staphylococcus saprophyticus subsp. bovis*	*ermB*	
AB137	*Staphylococcus epidermidis*	*blaZ*	*icaD*
AB138	*Staphylococcus cohnii subsp. urealyticus*	*icaA*	
AB139	*Staphylococcus epidermidis*	*blaZ, ermA*	*icaA, icaB, icaC, icaD, icaR*
AB148	*Staphylococcus haemolyticus*	*mecA*	
AB149	*Staphylococcus saprophyticus subsp. bovis*	*blaZ, ermB,*	*icaD, icaR*
AB155	*Staphylococcus hominis*	*blaZ*	*icaR*
AB156	*Staphylococcus cohnii subsp. ureolyticus*	*blaZ*	
AB158	*Staphylococcus capitis*	*blaZ*	*icaA, icaR*
AB160	*Staphylococcus hominis*	*blaZ, ermC*	
AB161	*Staphylococcus epidermidis*	*blaZ, ermA, ermC*	
AB163	*Staphylococcus cohnii subsp. urealyticus*	*blaZ, ermA, ermC*	
AB164	*Staphylococcus aureus*	*blaZ, ermA*	*icaD*
AB165	*Staphylococcus aureus*	*mecA, blaZ, ermA*	*icaD, icaR*
AB167	*Staphylococcus saprophyticus subsp. bovis*	*blaZ, ermC*	*icaR*
AB168[a]	*Staphylococcus capitis*		*icaA*
AB169	*Staphylococcus kloosii*	*blaZ, ermA*	

Table 4 continued

Isolate code	Species	Antibiotic resistance gene	Biofilm synthesis gene
AB170	*Staphylococcus hominis*	*mecA, blaZ, ermA*	*icaC, icaR*
AB171	*Staphylococcus aureus*	*blaZ, ermA, ermC*	*icaD, icaR*
AB172	*Staphylococcus saprophyticus subsp. bovis*	*ermA*	*icaR*
AB173	*Staphylococcus pasteuri*	*mecA, blaZ*	
AB176	*Staphylococcus epidermidis*	*blaZ*	*icaR*
AB177	*Staphylococcus kloosii*	*blaZ*	
AB181	*Staphylococcus saprophyticus subsp. bovis*	*blaZ*	*icaR*
AB220	*Staphylococcus kloosii*	*blaZ, ermC*	
AB221	*Staphylococcus cohnii*	*blaZ, ermC*	
AB222	*Staphylococcus hominis*	*blaZ, ermC*	
AB223	*Staphylococcus hominis*	*mecA, blaZ, ermC*	
AB225	*Staphylococcus aureus*	*ermA, blaZ*	
AB226	*Staphylococcus epidermidis*	*blaZ, ermC*	
AB227	*Staphylococcus capitis*	*blaZ*	
AB229	*Staphylococcus epidermidis*	*blaZ, ermC*	
AB230	*Staphylococcus saprophyticus subsp. bovis*	*blaZ, ermC*	
AB231	*Staphylococcus cohnii*	*ermA, blaZ*	
AB232	*Staphylococcus epidermidis*	*blaZ*	
AB233	*Staphylococcus epidermidis*	*blaZ*	
AB234	*Staphylococcus pasteuri*	*blaZ*	
AB236	*Staphylococcus aureus*	*ermA, blaZ*	
AB239	*Staphylococcus aureus*	*ermA, blaZ*	
AB250	*Staphylococcus capitis*	*blaZ*	
AB300	*Staphylococcus* sp.	*blaZ*	*icaA, icaR*
AB301	*Staphylococcus epidermidis*	*blaZ, ermC*	*icaA, icaB, icaD, icaR*
AB302	*Staphylococcus* sp.	*blaZ*	
AB303	*Staphylococcus hominis*	*blaZ,*	*icaA, icaR*
AB304	*Staphylococcus epidermidis*	*blaZ,*	
AB305	*Staphylococcus epidermidis*		*icaA, icaB, icaD, icaR*
AB306	*Staphylococcus* sp.	*ermC*	
AB307	*Staphylococcus epidermidis*	*blaZ*	*icaA, icaD, icaR*
AB308	*Staphylococcus cohnii*	*ermC*	*icaR*
AB310	*Staphylococcus* sp.		*icaA*
AB311	*Staphylococcus epidermidis*	*ermC*	*icaR*
AB312	*Staphylococcus* sp.		*icaA, icaR*
AB313	*Staphylococcus cohnii*	*ermC*	*icaR*
AB315[a]	*Staphylococcus haemolyticus*	*blaZ*	*icaR*
AB316[a]	*Staphylococcus* sp.		*icaA, icaR*
AB317[a]	*Staphylococcus saprophyticus*		*icaA*
AB318[a]	*Staphylococcus haemolyticus*	*blaZ*	*icaB, icaR*
AB319[a]	*Staphylococcus arlettae*		*icaA, icaR*
AB320[a]	*Staphylococcus* sp.		*icaB*
AB321	*Staphylococcus epidermidis*	*blaZ, ermC,*	*icaA, icaC, icaD, icaR*
AB322	*Staphylococcus epidermidis*	*blaZ*	*icaA, icaB, icaC, icaD, icaR*
AB325	*Staphylococcus epidermidis*	*blaZ,*	*icaA*

Table 4 continued

Isolate code	Species	Antibiotic resistance gene	Biofilm synthesis gene
AB328	*Staphylococcus capitis*		*icaA, icaR*
AB329	*Staphylococcus hominis*	*blaZ*	
AB330[a]	*Staphylococcus haemolyticus*	*mecA, blaZ*	
AB332	*Staphylococcus epidermidis*	*blaZ*	*icaA, icaB, icaC, icaD, icaR*
AB333	*Staphylococcus epidermidis*	*ermC*	
AB334	*Staphylococcus epidermidis*	*ermC*	
AB335	*Staphylococcus saprophyticus*		*icaR*
AB336	*Staphylococcus hominis*	*blaZ*	*icaB*
AB338	*Staphylococcus hominis*	*ermA, ermB*	
AB341[a]	*Staphylococcus capitis*	*blaZ*	*icaR*
AB342	*Staphylococcus saprophyticus subsp. saprophyticus*	*blaZ*	
AB343	*Staphylococcus saprophyticus subsp. saprophyticus*	*blaZ*	
AB345	*Staphylococcus sciuri*	*mecA, blaZ, ermC*	*icaD*
AB346	*Staphylococcus* sp.	*mecA, ermA*	
AB349	*Staphylococcus epidermidis*	*mecA*	*icaA, icaB, icaC, icaD, icaR*
AB350	*Staphylococcus epidermidis*		*icaA, icaB, icaC, icaD, icaR*
AB351	*Staphylococcus epidermidis*		*icaA, icaB, icaC, icaD, icaR*
AB352	*Staphylococcus haemolyticus*	*blaZ*	
AB355	*Staphylococcus epidermidis*	*blaZ, ermA*	*icaD*
AB357	*Staphylococcus* sp.	*blaZ*	
AB359	*Staphylococcus epidermidis*	*mecA*	*icaA, icaB, icaC, icaD, icaR*
AB360	*Staphylococcus epidermidis*		*icaA, icaB, icaC, icaD, icaR*
AB362	*Staphylococcus* sp.	*mecA*	
AB363	*Staphylococcus pasteuri*	*blaZ*	*icaA, icaB*
AB365	*Staphylococcus hominis*	*blaZ*	
AB366	*Staphylococcus hominis*		*icaA*
AB367[a]	*Staphylococcus hominis*	*blaZ*	*icaA*
AB368	*Staphylococcus haemolyticus*	*mecA, blaZ*	
AB369	*Staphylococcus epidermidis*	*blaZ*	
AB370	*Staphylococcus hominis*	*mecA, blaZ*	
AB371	*Staphylococcus hominis*	*blaZ*	*icaA, icaB, icaR*
AB373	*Staphylococcus warneri*	*blaZ, ermC*	
AB374	*Staphylococcus arlettae*	*ermC*	
AB375	*Staphylococcus warneri*	*blaZ, ermA, ermC*	
AB376	*Staphylococcus saprophyticus*	*ermA, ermC*	
AB377[a]	*Staphylococcus saprophyticus*	*ermC*	
AB378[a]	*Staphylococcus* sp.	*blaZ*	
AB379[a]	*Staphylococcus haemolyticus*	*blaZ*	
AB380	*Staphylococcus cohnii*	*ermB*	
AB382	*Staphylococcus hominis*	*blaZ, ermC*	
AB383	*Staphylococcus epidermidis*	*blaZ, ermC*	
AB385	*Staphylococcus cohnii*	*blaZ, ermC*	
AB386	*Staphylococcus cohnii*	*blaZ, ermB, ermC*	
AB387	*Staphylococcus cohnii*	*blaZ, ermB, ermC*	
AB388	*Staphylococcus* sp.	*mecA, ermC*	

Table 4 continued

Isolate code	Species	Antibiotic resistance gene	Biofilm synthesis gene
AB391	*Staphylococcus epidermidis*	*mecA, blaZ*	
AB392	*Staphylococcus* sp.	*mecA, blaZ, ermA*	
AB393	*Staphylococcus warneri*	*mecA, blaZ, ermA*	
AB394	*Staphylococcus warneri*	*blaZ, ermA*	
AB395	*Staphylococcus* sp.	*mecA*	
AB396[a]	*Staphylococcus cohnii*	*ermC*	
AB398[a]	*Staphylococcus aureus*	*blaZ*	*icaR*
AB399[a]	*Staphylococcus aureus*	*blaZ, ermA*	*icaD, icaR*
AB400[a]	*Staphylococcus aureus*	*blaZ, ermB*	*icaD, icaR*
AB401	*Staphylococcus aureus*	*blaZ, ermB, ermC*	*icaD, icaR*
AB402	*Staphylococcus epidermidis*	*mecA, blaZ*	*icaR*
AB403	*Staphylococcus aureus*	*blaZ*	*icaR*
AB404[a]	*Staphylococcus aureus*	*blaZ, ermC,*	*icaD, icaR*
AB405	*Staphylococcus* sp.	*blaZ, ermC*	*icaA*
AB406[a]	*Staphylococcus aureus*	*blaZ, icaR*	
AB407[a]	*Staphylococcus aureus*	*blaZ, ermB*	*icaD, icaR*
AB408	*Staphylococcus aureus*	*blaZ, ermB*	*icaD, icaR*
AB409	*Staphylococcus aureus*	*blaZ*	*icaD, icaR*
AB410	*Staphylococcus arlettae*	*blaZ*	
AB413	*Staphylococcus aureus*	*blaZ*	
AB414	*Staphylococcus warneri*	*blaZ*	
AB417	*Staphylococcus cohnii*	*blaZ, ermC*	*icaA, icaR*
AB418	*Staphylococcus capitis*		*icaA, icaR*

AB Airborne bacteria

[a]Samples obtained from outdoor air, as recommended by RE 09/2003 ANVISA

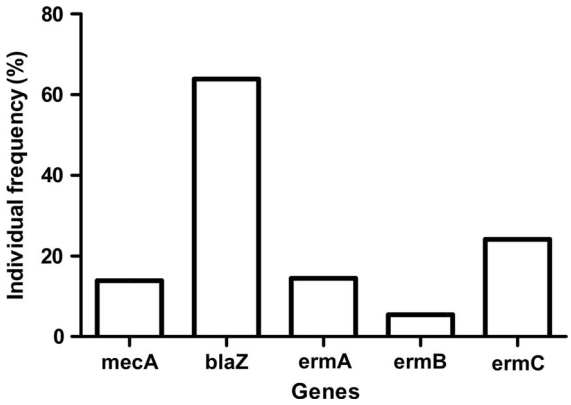

Fig. 4 Individual detection of antibiotic resistance genes in airborne *Staphylococcus* (n = 166)

other *Staphylococcus* species, they are rarely mentioned as part of the human microbiota (Rosenstein et al., 2008; Yu et al., 2008). This fact justifies the low detection of these isolates in our analysis. Notably, we report the first detection of the species *S. warneri*, *S. kloosii*, *S. carnosus*, *S. sciuri*, *S. arlettae*, and *S. gallinarum* indoor air contaminants in commercial aircraft.

Many factors are responsible for influencing bioaerosols' concentration in indoor air. Due to limitations that made it impossible to investigate other parameters, our analysis sought to verify only the influence of outdoor air as a source of airborne *Staphylococcus*. Thus, air collections were performed in open areas of the airport near the aircraft evaluated at landing. Our results suggest that the airborne

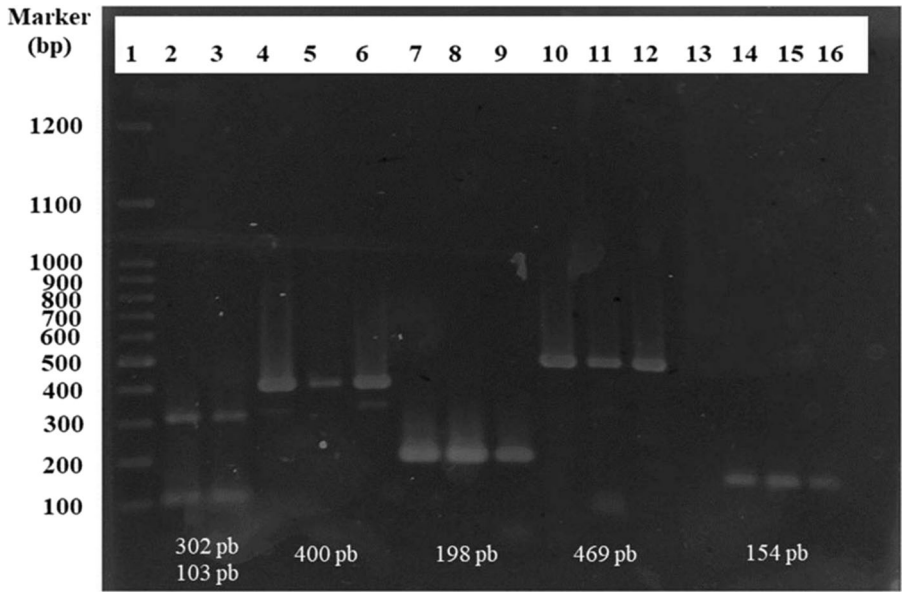

Fig. 5 Detection of *mecA* gene and icaADBC *locus* genes and icaR promoter region in airborne *Staphylococcus*. Electrophoresis on a 1.2% agarose gel. Lanes: 2–3, positive *ica*A (103 pb) and *ica*B (302 pb) genes; 4–6, positive *ica*C gene (400 pb); 7–9, positive *ica*D gene (198 pb); 10–12, positive *ica*R (469 pb) gene; 14–16, positive *mecA* gene

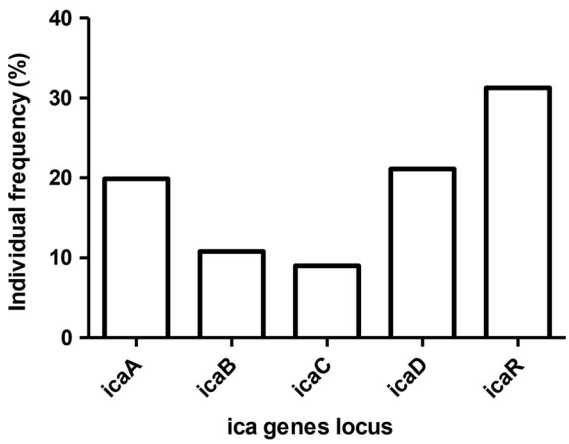

Fig. 6 Detection of icaADBC *locus* genes and the icaR promoter region in airborne *Staphylococcus*. (n = 166)

Staphylococcus concentrations in indoor air may not have been influenced by outdoor air, mainly because the number of indoor isolates was significantly higher than those obtained in outdoor air. These results differ from those found by Chegini et al. (2020) and Mirhoseini et al. (2020), who reported outdoor air as the primary source of bacterial bioaerosols for indoor air in kindergarten and hospital settings, respectively. In contrast with us, Marcovecchio and Perrino (2021a) found that an increase in the concentration of biological aerosols in indoor environments is closely related to the presence of internal sources, being human occupation the most important. Besides that, Marcovecchio and Perrino (2021b) observed that in crowded indoor environments, human occupation seems to be the main factor responsible for the increase in the concentration of indoor bioaerosols. This finding can be significant for an environment with a high concentration of people, such as an aircraft cabin. Although we have detected a few species in indoor and outdoor air, our results suggest other factors may contribute to the increased concentration of bacterial bioaerosols in the cabin environment. Thus, internal factors such as human occupation, handling of food in the cabin, and dust particles can have a greater significance than outdoor air. Since each indoor environment has characteristics and specific factors that act on bioaerosols' concentration, it is essential to elucidate the exact factors involved in the concentration and persistence of airborne bacteria in the cabin environment.

The presence of aerosols containing microorganisms that may cause infection within the cabin environment on aircraft warns of the exposure risks in this environment. Second scientific reports, almost all species identified in this study seem to be

associated with infections in humans (Al Hennawi et al., 2019; Natsis & Cohen, 2018), except for *S. carnosus* and *S. arlettae*. Like our results, McManus and Kelley (2005) evaluated bacterial contamination of surfaces inside commercial aircraft and verified the presence of species known to cause opportunistic infections in humans, including *S. epidermidis* and *S. hominis*. Fu et al. (2013) have also shown aerial exposure to other indoor air contaminants harmful to human health within the aircraft cabin environment. In this study, the authors verified the presence of allergens and microbial volatile organic compounds (MVOCs) related to respiratory problems. This diversity of airborne contaminants (mainly microorganisms) demonstrates the high susceptibility experienced by individuals in a confined environment such as an aircraft cabin, with a particular risk of those who have some immunological impairment. Our results also point to the need to implement a program to monitor indoor air quality within the aircraft cabin environment as an essential safety measure for human health.

In our analysis, we detected airborne *Staphylococcus* with antimicrobial resistance genes, including methicillin-resistant *Staphylococcus aureus* identification. Until this study was carried out, we found no research that reported the detection of antibiotic resistance genes in bacterial isolates obtained from the indoor air of commercial aircraft's cabin. Thus, our findings corroborate the evidence that indoor air can play an essential role in disseminating antibiotic resistance genes and brings proof about the possibility that aircraft air-conditioning systems serve as a source of airborne multidrug-resistant bacteria for the cabin environment.

Of the investigated antibiotic resistance genes, only *mecC* and *vanA* genes were not found. For the *mecC* gene, this absence was already expected, mainly because it was recently discovered. There are still few reports in the literature about its detection (Kerschner et al., 2015). Moreover, no description of this gene's presence in Brazil had been made by the time this research was conducted. Regarding the *vanA* gene, was reported its occurrence in methicillin-susceptible *Staphylococcus aureus* and MRSA isolates in Brazil. However, its occurrence seems to be an infrequent event with few literature descriptions (Damasco et al., 2019; Panesso et al., 2015). To date, there has been no report of the presence of these genes in airborne *Staphylococcus*. Although we have not detected these genes in our isolates, their occurrence in the cabin environment cannot be ruled out. This is due to the fact that antibiotic resistance genes (ARGs) can be found in bacteria present in samples other than air. Recently, Heß et al. (2019) verified a great diversity and quantity of ARGs in airplane sewage. Like us, the authors did not find the *VanA* and *mecC* genes, but they detected genes involved in Tetracycline resistance, which is also used to treat *Staphylococcus* infections. In disagreement with us, Petersen et al. (2015) performed a metagenomic analysis of toilet waste from long-distance flights and verified the presence of genes involved in resistance to glycopeptides, which is the class vancomycin to belong. Thus, ARGs in the cabin environment can involve several sources, with indoor air being the most effective route in disseminating and transmitting multidrug-resistant bacteria to an exposed susceptible individual. *S. gallinarum* and *S. carnosus* were not positive for carrying any of the screened genes, which can be explained by the fact that only one isolate was obtained for each species.

We report for the first time the detection of MRSA and MR-CoNS in indoor air samples collected on commercial aircraft. It had been already observed in other artificially air-conditioned environments. However, it had not yet been done in the cabin environment. Like us, Kumar and Goel (2016) also verified MRSA and MR-CoNS in residential houses' indoor air. On the other hand, Solomon et al. (2017) identified only MRSA among airborne *Staphylococcus* isolated from indoor air in the hospital environment. The detection of airborne MRSA and MR-CoNS in commercial aircraft demonstrates how decisive air can favor outbreaks of infections in non-hospital environments. Also, surfaces as a critical vector of the environmental transmission of multidrug-resistant bacteria have been reported in the literature (Fritz et al. 2020). In the indoor air quality context, they can serve as important MRSA reservoirs and contribute significantly to their aerosolization. In this way, MRSA can be disseminated within the cabin environment by routes other than air, for example, from the sedimentation of bioaerosols on surfaces or association with dust particles. Based on the above, it is crucial to consider the air quality monitoring and hygiene of commercial aircraft as essential measures to ensure safety and minimize health risks by exposure to airborne MRSA within the cabin environment.

In infections by methicillin-susceptible *Staphylococcus*, the use of penicillin is the most widely used therapeutic option, primarily because of the advantages over other drugs such as glycopeptides and other β-lactams. Detection of the *blaZ* gene already was reported in clinical and environmental isolates of *Staphylococcus* (Okiki et al., 2020). However, this is the first report in isolates obtained from indoor air in commercial aircraft. Even for other indoor environments, investigations about this gene in airborne *Staphylococcus* have not been described. Only Creamer et al. (2014) reported detecting the *blaZ* gene in airborne isolates obtained from air samples from hospital wards. In contrast with our survey, the authors conducted analyses focusing only on *S. aureus*. We found that CoNS also carried the *blaZ* gene. Besides that, genes involved in cross-resistance to macrolides, lincosamides, and streptogramin B (MLSB) were found in our screening. All MLSB resistance genes investigated were detected in airborne *Staphylococcus* isolates, with *ermC* and *ermA* being the most frequently detected. The low detection of *ermB* corroborates the hypothesis that this gene is much more frequent in other Gram-positive bacteria than in *Staphylococcus* (Jensen et al., 1999). Lenart-Boroń et al. (2017) evaluated the antimicrobial resistance profile in *Staphylococcus* sp. obtained from air samples from living spaces and reported the detection of *erm* genes in the identified bacteria. Unlike our analyses, they verify *ermB* as the most frequent and *ermA* as the least frequent in their samples. In commercial aircraft, Petersen et al. (2015) also described the abundant occurrence of *ermA*, *ermB*, and *ermC* genes based on metagenomic analyses of toilet waste from long-distance flights. MRSA and MR-CoNS carried *blaZ* and *erm* genes, proving the importance of the environment as a possible source of contamination by multidrug-resistant bacteria. These results emphasize the significance of air travel in the spread of drug-resistant bacteria.

In the context of serious infections caused by *Staphylococcus* sp. and antibiotic resistance, biofilm formation is a significant public health challenge. In this research, we investigated the occurrence of genetic determinants involved in the synthesis of biofilms in airborne *Staphylococcus* in the cabin environment. Although we have detected the presence of *ica* genes in several species identified here, only in some isolates of *S. epidermidis* was found the joint detection of the *ica*A and *ica*D genes. These genes are critical for the biofilm formation in this bacterial group, and their detection in aerial isolates may be relevant in the context of chronic infections. Unlike other reports, we did not observe detection of *ica*A and *ica*D genes in the other identified species, which has already been seen in *S. aureus* and *S. haemolyticus* (Nourbakhsh & Namvar, 2016; Pinheiro et al., 2016). Since few samples were positive for biofilm formation via *ica* genes, other mechanisms may be involved in negative isolates. It seems to be closely related to each *Staphylococcus* isolate's specificity (Archer et al., 2011; Gotz, 2002). Moreover, environmental factors can also be important in stimulating the expression of genes related to biofilm synthesis.

A limited amount of studies has sought to investigate the presence of genes for biofilm formation in airborne bacteria. Our study is the first to make this detection in isolates obtained from the aircraft cabin environment. Seo et al. (2008) detected genes related to biofilm formation in *S. epidermidis*, *S. aureus,* and *S. hominis* obtained from indoor air in public environments. They found that *S. epidermidis* was the main species carrying the *ica*A and *ica*D genes, equal in our results. The absence of detection of *ica*A and *ica*D in other species of *Staphylococcus* in our work compared to the study by Seo et al. (2008) may be due to the presence of a more significant number of sources of this bacterium for indoor air, as well as the existence of many more stimulating factors the biofilm formation. In the results of Botelho et al. (2012) and Rahman (2019), *S. epidermidis* collected from indoor air in hospitals and car air conditioners were also positive for detecting *ica*A and *ica*D genes. The detection of the *ica*A, *ica*D, and *mecA* genes was verified in a few isolates of *S. epidermidis* and highlights the importance of monitoring indoor air quality as an indispensable tool in biological knowledge risks within the cabin environment.

This study contains limitations that may have impacted the achievement of a more extensive set of data and restricted our results' analysis. These limitations were mainly due to the airport administration's and airlines restrictions. During data collection, we were unable to investigate further which factors could influence bioaerosols' presence in the cabin environment because of the limited time that we could access the aircraft yard. This area is an extremely restricted and high-security location. Our permanence in the

aircraft for the collection was also monitored concerning the time we could be on board. Due to the lack of national and international air quality legislation in commercial aircraft cabins, the in-flight collection was not possible because airlines did not grant permission. There were differences related to indoor and outdoor air collections. For a collection day, we sampled up to five aircraft and only one sample of outdoor air. Thus, precise comparison between outdoor air and its influence on microbial concentration inside the aircraft may have been underestimated. An excellent experimental design would include outdoor air collections immediately after the indoor air collection inside the aircraft. Although we consider the limitations that could not be controlled, our work further demonstrates the importance of monitoring indoor air quality as an instrument for preventing the spread of airborne infections. Also, it points out needs to discuss IAQ issues in civil aviation, similarly to what occurs in other artificially air-conditioned environments.

5 Conclusion

In this survey, we report indoor air contamination by a wide range of airborne *Staphylococcus* species in commercial aircraft, including the presence of species that may be critical human pathogens. Of all those identified, *S. epidermidis* and *S. hominis* were the most frequent. The occurrence of the clinically significant *S. aureus* was also observed in some of the evaluated aircraft. Antibiotic resistance genes detected in airborne *Staphylococcus* were: *mecA* (resistance to methicillin/oxacillin), *blaZ* (resistance to penicillins), and those involved MLSB resistance. The mecA gene was found in coagulase-negative *Staphylococcus*, but mainly in *S. aureus* isolates, characterizing MRSA strains. Genetic determinants of *ica* operon related to biofilm formation were also found in airborne *Staphylococcus*, especially the joint detection of *ica*A and *ica*D genes only in *S. epidermidis*.

The evidence presented here is expected to contribute to discussions in indoor air quality in commercial aviation to consider establishing regulatory standards to reduce the risks of airborne pathogen transmission and the spread of antibiotic resistance genes through air travel. From these results, investigations involving the research of the other airborne bacteria, airborne fungi, and other indoor air contaminants (e.g., mycotoxins, allergens, and mVOCs) in commercial aircraft should also be considered.

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Authors' contributions EASF and JPMN took part in conception and design; JPMN, DB, MAA, and FLF involved in methodology and data analysis; JPMN and EASF participated in writing—original draft preparation; LA and LAF took part in writing—review and editing. All authors read and approved the final manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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