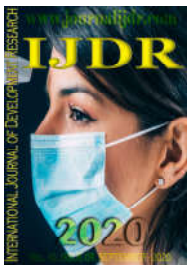
ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 10, Issue, 09, pp. 40883-40890, September, 2020
https://doi.org/10.37118/ijdr.20077.09.2020



RESEARCH ARTICLE OPEN ACCESS

ANTIBACTERIAL ACTIVITY OF RED PROPOLIS FROM ALAGOAS AGAINST *STAPHYLOCOCCUS* SPECIES ISOLATED IN AN INTENSIVE CARE UNIT

Rodrigo J. N. Calumby[1,*], Teresa L. G. F. Lôbo[1], Rafael A. S. Silva[1], Jeniffer M. D. de Freitas[2], Rossana T. F. Moreira[3], Lara M. de Almeida[1], Ticiano G. do Nascimento[1], Luciano A. M. Grillo[1] and Valter Alvino[1]

[1]Instituto de Ciências Farmacêuticas, Universidade Federal de Alagoas, Maceió – AL,Brazil.
[2]Faculdade de Nutrição, Universidade Federal de Alagoas, Maceió – AL, Brazil.
[3]Escola de Enfermagem, Universidade Federal de Alagoas, Maceió – AL, Brazil.

ARTICLE INFO

Article History:
Received 14th June 2020
Received in revised form
26th July 2020
Accepted 11th August 2020
Published online 30th September 2020

Key Words:
Red propolis from Alagoas,
Staphylococcus,
MRSA, Intensive care unit.

Corresponding author:
Rodrigo J. N. Calumby

ABSTRACT

The objective of this study was to evaluate the antibacterial activity of the Red Propolis from Alagoas (RPA) against *Staphylococcus* species isolated from air conditioner filters and surfaces of an intensive care unit in a tertiary-care public hospital in Maceió (Alagoas State). Sterile swabs were used for sample collection. Bacteria were grown in brain heart infusion (BHI) agar at 37^0C (24 hours), and presumptively identified by conventional biochemical tests. Antimicrobial susceptibility was determined using the disk diffusion method. Fresh propolis was macerated to obtain the Hydroalcoholic Extract (HAE), which was fractionated using the liquid-liquid partition method, resulting in the Medium Polarity (MPF) and Medium-High Polarity (MHPF) Fractions. The antimicrobial activity of the RPA was evaluated using the broth microdilution method. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined. HAE and fractions showed activity against staphylococcal strains (MIC, 64-1024 µg mL^{-1}; MBC, 256-2048 µg mL^{-1}). MPF showed lower MIC values. Our data show an antibacterial potential of the RPA against clinical isolates of *Staphylococcus*, including Methicillin-Resistant *S. aureus* (MRSA) strains.

Citation: ***Rodrigo J. N. Calumby, Teresa L. G. F. Lôbo, Rafael A. S. Silva, Jeniffer M. D. de Freitas, Rossana T. F. Moreira et al.*** "Antibacterial activity of red propolis from alagoas against staphylococcus species isolated in an intensive care unit*", International Journal of Development Research*, 10, (09), 40883-40890.

INTRODUCTION

Health Care-Related Infections (HAIs) represent a major public health concern, increasing mortality rates, affecting the hospitalization period of affected patients, in addition to increasing the costs involved in this hospitalization (Padoveze; Fortaleza, 2014). Air conditioners and contact surfaces of hospital environments are recognized as reservoirs of infectious microorganisms and present a great risk for the spread of multi-resistant pathogens (Gebel *et al*., 2013; Weber *et al*., 2013; Han *et al*., 2015). Periodic cleaning of air conditioner filters and disinfection of surfaces is extremely important for infection control in Intensive Care Units (ICUs) (Carling; Bartley, 2010; Santos Junior *et al*., 2018), as immunocompromised patients undergo invasive procedures becoming susceptible to opportunistic infections (Streit *et al*., 2004; Zilahi *et al*., 2016; MacVane *et al*., 2017). In particular, contact surfaces must be cleaned and disinfected with highly effective antimicrobial agents (Vickery *et al*., 2012). Studies investigating cleaning interventions have shown that approximately 5 to 30% of surfaces remain potentially contaminated due to the inability of existing detergent and disinfectant formulations in avoiding biofilm formation (Ramm *et al*., 2015; Sattar *et al*., 2015), indicating the need for new strategies for cleaning and disinfecting. The Red Propolis of Alagoas (RPA) is a complex material composed of resinous exudate produced by bees (*Apis mellifera*). It is found in mangrove areas of the State of Alagoas, Brazil, and its main botanical source is *Dalbergia ecastophyllum* (L.) Taub. (Fabaceae), popularly known as "rabo-de-bugio" (Alencar *et al*., 2007; Libério *et al*., 2009). RPA is classified as the 13th type of Brazilian propolis and its biological activity differs from the other types due to the presence of phenolic compounds, such as isoflavones, chalcones, pterocarpanes, terpenes, polyprenylated benzophenones (guttiferones), and condensed tannins (Daugsch, *et al*., 2008; Bueno-Silva *et al*.,

2013). Recent studies have highlighted its antimicrobial activity against several microorganisms, including Gram-positive and Gram-negative bacteria (Regueira-Neto *et al*., 2019; Nascimento *et al*., 2019; Miranda *et al*., 2019). The RPA also presents other pharmacological properties, including antioxidant (Reis *et al*., 2019), antifungal (Pippi *et al*., 2015), antiviral (Silva-Beltrán *et al*., 2019) and anti-inflammatory (Bueno-Silva *et al*., 2017) activities. Given the above, the objective of this study was to evaluate the antibacterial activity of RPA against *Staphylococcus* species isolated from an ICU in a tertiary-care public hospital in Maceió (Alagoas).

MATERIALS AND METHODS

Sample Collection

This study was conducted in the General ICU in a tertiary-care public hospital in Maceió (Alagoas State), which has 8 beds for young and adult patients. Monthly collections of air conditioner filter and surface samples (entrance door, serum support, patient's bed, and sink) were carried out from February to April, 2018. Samples were collected in an area of 20 cm^2 of each site using sterile swabs moistened in 0.9% saline solution, which were seeded by radial spreading in Petri dishes containing Brain Heart Infusion (BHI) agar. Then, the plates were incubated at ± 35°C for 24 hours. After the time the cultures remained in the microbiological oven, the growth of microorganisms was detected by counting Colony Forming Units (CFU). Gram stain was used to classify bacteria based on morphotintorial characteristics. For identification of Gram-positive cocci, biochemical tests were performed as follows: catalase, coagulase, DNase, 6.5% NaCl, esculin bile, and susceptibility to novobiocin, bacitracin and optoquine (Murray *et al*., 2017). Gram-negative and Gram-positive Bacilli isolated at the sampled sites were not identified, since this study focused on Gram-positive cocci, especially *Staphylococcus* spp., which are considered important causes of contamination in hospital environments.

Antimicrobial Susceptibility Test (TSA): After presumptive identification of *Staphylococcus*, the most prevalent species were subjected to the antimicrobial susceptibility test (TSA). The Agar Diffusion Disc method was performed according to the guidelines of the Clinical Laboratory Standards Institute (CLSI, 2019). The following antibiotics were tested: penicillin (10µg), gentamicin (10µg), ciprofloxacin (5µg), vancomycin (50µg), sulfamethoxazole-trimethoprim (25µg), ceftriaxone (30µg), chloramphenicol (30µg), tetracycline (30µg), cefoxitin (30µg), and oxacillin (5µg).

Hydroalcoholic extract and fractions of red propolis from Alagoas: A sample of red propolis from the apiary Ilha do Porto was used to obtain the hydroalcoholic extract and fractions. The apiary was located in a mangrove area in Marechal Deodoro (AL), in the Northeast Region of Brazil. Obtaining the hydroalcoholic extract and typified fraction was based on the methodology used by Alencar *et al*. (2007). The ground fresh propolis was subjected to cold extraction (maceration) and left to stand for 48 hours at room temperature, using 96 ° GL ethanol as a solvent. Three washes were performed with ethanol to exhaust the extraction of the compounds. The filtration of this solution was performed on filter paper, using a vacuum pump. Then, the filtrate was concentrated in a rotary evaporator, and after evaporation of the solvent, the Propolis Crude Extract (PCE) was obtained.

At this stage, 10% of distilled water was added to obtain the Propolis Hydroalcoholic Extract (HAE). The extract fractionation was carried out using the liquid-liquid extraction technique, in a separating funnel, so that the HAE was partitioned with solvents of increasing polarity: hexane and ethyl acetate, after evaporation of the solvents in a rotary apparatus at reduced pressure what they were obtained the Low Polarity (LPF), Medium Polarity (MPF), Medium-High Polarity (MHPF) and High Polarity (HPF) fractions. For microbiological assays, MPF and MHPF were selected, as they have a higher content of flavonoids in previous chromatographic analysis and have demonstrated better antibacterial activity in preliminary tests against ATCC strains. The samples were solubilized in Dimethyl Sulfoxide (DMSO) 2%, obtaining a concentration of 4096 µg mL^{-1}.

Antimicrobial activity of RPA: The selected extracts and fractions of RPA were tested against *Staphylococcus aureus* and *Staphylococcus epidermidis* isolates using the broth microdilution assay. Minimum Inhibitory Concentration (MIC) was determined according to the document M07 of the Clinical Laboratory Standards Institute (CLSI, 2018). The *S. aureus* strain ATCC 25923 was used as a control of the experiment. The Minimum Bactericidal Concentration (MBC) was determined based on the methodology of Santurio *et al*. (2007). From the wells in which there was no visible bacterial growth, in the microdilution assay, an aliquot of 10 µL was removed and then grown on the surface of the Mueller Hinton Agar (MHA) contained in Petri dishes. Subsequently, the plates were incubated at 35ºC and, after 24 hours, the MBC was defined as the lowest concentration of the extract in a study capable of promoting the death of the inoculum. MIC and MBC trials were performed in triplicate.

RESULTS AND DISCUSSION

Bacteria isolated from the ICU: Quantitative analysis of colonies isolated from air conditioner filters and surfaces showed 190 Colony Forming Units (CFU) during the three collections. The air conditioner with 94 (49.5%) CFU, followed by the surface of the ICU entrance door with 45 CFU (23.7%) corresponded to the main sources of contamination, as shown in Table 1. The air conditioner is recognized as a source of propagation of pathogens (Quadros *et al*., 2009; Silva *et al*., 2013; Eslami *et al*., 2016; Libert *et al*., 2019), through the production of aerosols, by the transmission of particles from the contaminated filters, due to the low rate of renewal and exhaustion of the air or even the condensation trays, where biofilms are often formed which are capable of promoting the growth of microorganisms (Nunes, 2005; Mobin; Salmito, 2006). According to Quadros *et al*. (2009), air quality in air-conditioned environments has a direct and significant influence on the speed of recovery of patients and the occurrence of infections.

Table 1. Total CFU distribution of bacteria isolated from air conditioner filters and ICU surfaces at a tertiary-care public hospital in Maceió (Alagoas State)

SAMPLES		CFU	%
Surfaces	Patient's bed	27	14.2
	Serum support	4	2.1
	Sink	20	10.5
	Entrance door	45	23.7
Equipment	Air conditioner	94	49.5
TOTAL		190	100.0

Table 2. Distribution of CFU according to the microorganism isolated in the ICU of a tertiary-care public hospital in Maceió (Alagoas State)

MICROORGANISMS	CFU	%
Staphylococcus aureus	123	64,7
Staphylococcus epidermidis	49	25,7
Staphylococcus saprophyticus	2	1,1
Enterococcus spp.	1	0,5
Gram-negative bacilli	10	5,3
Gram-positive bacilli	3	1,6
Unidentified	2	1,1
TOTAL	190	100,0

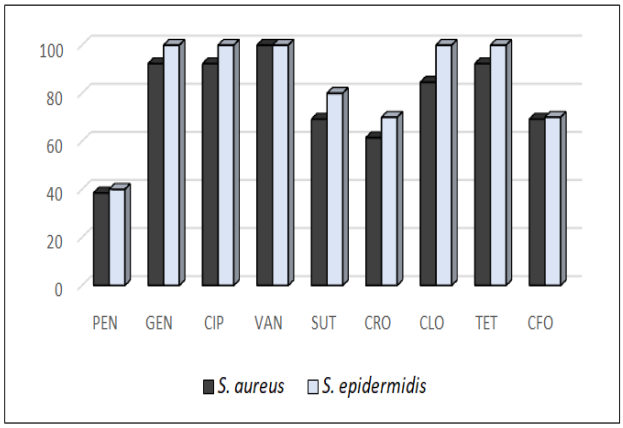


Figure 1. Antimicrobial susceptibility profile of *S. aureus* (n=13) and *S. epidermidis* (n=10) isolated from the ICU of a tertiary-care public hospital in Maceió (Alagoas State). PEN = Penicillin; GEN = Gentamycin; CIP = Ciprofloxacin; VAN = Vancomycin; SUT = Sulfamethoxazole-Trimethoprim; CRO = Ceftriaxone; CLO = Chloramphenicol; TET = Tetracycline and CFO = Cefoxitin.

A considerable percentage of microbial contamination was also found in the ICU entrance door (45 CFU / 23.7%) and in a patient's bed (27 CFU / 14.2%), which can serve as a vehicle for contamination among professionals, visitors, and patients (Andrade *et al*., 2006). In this sense, the cross-transmission of microorganisms from surfaces can play a significant role in colonization and infections acquired in the ICU. Contamination can result from the hands of healthcare professionals or direct spillage of bacteria by patients, capable of surviving for several months on dry surfaces (Russotto *et al*., 2015). In this way, the importance of frequent disinfection of surfaces is emphasized. Besides, the correct use of gloves by professionals is essential, as well as adequate hygiene of the patient and the hands of family members and visitors, to minimize the appearance of HAIs (Oliveira; Damasceno, 2010). Among the 190 CFUs isolated, 175 (92.1%) were identified as Gram-positive cocci, 10 (5.3%) as Gram-negative bacilli and 3 (1.6%) as Gram-positive bacilli. Among Gram-positive cocci, 123 (64.7%) were found to be *Staphylococcus aureus*, 49 (25.7%) *Staphylococcus epidermidis*, 2 *Staphylococcus saprophyticus* (1.1%) and 1 *Enterococcus* spp. (0.5%), as shown in Table 2. Gram-positive bacteria, especially cocci, are microorganisms commonly isolated on hospital surfaces (Kramer *et al*., 2006; Gomes *et al*., 2016), corroborating the findings of this study. This fact is related to the colonization of these microorganisms, both in the skin or mucous membranes of humans and animals and in an environment where there is nature and/or inanimate objects, being able to survive for months on surfaces (Murray *et al*., 2017). Staphylococci, especially *Staphylococcus aureus*, are important bacteria associated with HAIs. *S. aureus* is frequently inoculated during invasive procedures or transmitted by the health team, and this situation is aggravated by the emergence of endemic multidrug-resistant strains in the hospital environment, especially in the ICU (Renner; Carvalho, 2013). Staphylococci constitute the skin microbiota, and can also cause opportunistic infections, due to the patient's immune deficiency, intracellular destruction, as in the case of burns, surgical wounds, presence of long-term catheters, suture threads, surgical prostheses, chronic-degenerative diseases, or even in cases of the prolonged use of antimicrobial agents (Siboo *et al*., 2001; Procop *et al*., 2018). To assess the susceptibility profile to antimicrobials, a total of 13 different strains of *S. aureus* and 10 of *S. epidermidis* were characterized. All isolates were vancomycin susceptible (100%). Nevertheless, only 5 (38.5%) isolates of *S. aureus* and 4 (40.0%) strains of *S. epidermidis* were susceptible to penicillin, in all collected sites. Four MRSA (Methicillin-resistant *Staphylococcus aureus*) strains were also identified. One of them was isolated from the air conditioner, 1 from the patient's bed, 1 from the serum support and 1 from the sink (Figure 1).

In Brazil, resistance to penicillin in *S. aureus* and *S. epidermidis* have been found in more than 80% of the isolates in both healthcare and community, and the use of this antimicrobial agent is no longer recommended for the treatment of staphylococcal infections (Boretti *et al*., 2014). Penicillin resistance occurs due to the production of beta-lactamases, which are widely spread among *Staphylococcus* species (Deng *et al*., 2015). In addition, these microorganisms have shown a high percentage of resistance to methicillin in hospitals in Brazil (Bride *et al*., 2019). ICU's are considered epicenters of bacterial resistance, becoming the main source of outbreaks of multidrug-resistant bacteria responsible for HAIs (Oliveira; Silva, 2008). In a study conducted by Renner e Carvalho (2013), aiming to evaluate the presence of microorganisms on surfaces of the adult ICU of a hospital in Vale do Rio Pardo - RS, Brazil, the resistance to penicillin was found in 93% of coagulase-negative *Staphylococcus* and 80% of strains of *S. aureus*. Regarding MRSA, epidemiological studies have shown that this microorganism is endemic in most hospitals in the world, raising the significant level of morbidity and mortality, especially in ICU patients (Ferreira *et al*., 2011; Cabrera *et al*., 2020; Chen *et al*., 2020; Mehta *et al*., 2020). MRSA contagion in internal patients occurs through the hands of health professionals and, although measures that improve adherence to hand hygiene are being implemented, cross-contamination is still a frequent problem (Alvarez *et al*. 2010). The methicillin resistance in *S. aureus* is related to the alteration of the penicillin-binding protein (PBP) PBP2a or PBP2´, which has low affinity for methicillin and other beta-lactams, thus preventing the arrival of antibiotics, generating a resistance pattern (Chambers; Deleo, 2009; Kumar *et al*., 2014). PBP2a is encoded by the *mec*A gene, which is carried by a mobile genetic element called Staphylococcal Chromosomal Cassette (SCC*mec*) (Kondo *et al*., 2001; Kondo *et al*., 2009; Chambers; Deleo, 2009; Ortíz-Gil *et al*., 2020).

Antimicrobial activity of RPA: Table 3 shows the MIC and MBC values using the hydroalcoholic extract and RPA fractions in the *Staphylococcus* species. MIC ranged from 64 to 1024 µg mL^{-1}, with the lowest value observed for the MPF against a MRSA strain. MBC ranged from 256 to 2048 µg mL^{-1} and, as for MIC, an MPF showed strong inhibition on an MRSA strain (256 µg mL^{-1}).

Table 3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of hydroalcoholic extracts and fractions of red propolis from Alagoas against *Staphylococcus* spp. isolates

Species (samples)	Extract and Fractions (MIC[1]/MBC[2])					
	HAE*		MPF*		MHPF*	
S. aureus (n=9)	512	1024	128	512	256	1024
S. epidermidis (n=10)	256	1024	128	512	512	1024
S. saprophyticus (n=2)	1024	NO	256	1024	512	2048
MRSA (n=4)	1024	NO	64	256	256	2048
S. aureus (ATCC 25923)	256	2048	128	512	256	1024

[1,2] in μg mL $^{-1}$.*The values presented correspond to the best results obtained from MIC and MBC for the strains evaluated.NO = Not observed.

Several studies have demonstrated action of propolis on bacteria, especially Gram-positive bacteria (Lopez *et al*., 2015; Dantas Silva *et al*., 2017; Rufatto *et al*., 2018; Silva *et al*., 2019; Regueira-Neto *et al*., 2019), since Gram-negative bacteria have a chemically more complex cell wall and a higher lipid content, which in some way hinders the action of the active components of propolis (Lustosa *et al*., 2008). Despite the differences in the composition of propolis, studies at different times and in different regions have demonstrated its antimicrobial activity (Monzote *et al*., 2012; Machado *et al*., 2016; Devequi-Nunes *et al*., 2018), which is attributed to substances derived from plants collected for its production.

Therefore, although it is a product of animal origin, some chemical compounds of propolis are derived from the botanical source used by bees, especially those with biological action (Pereira *et al*., 2015). In research by Nina *et al*. (2015), methanolic extracts from 19 samples of propolis produced in the Maule Region, in Chile, were tested for the antimicrobial effect against bacteria of clinical importance, with inhibitions of *S. aureus* being observed in samples ranging from 62.5 to 1000 μg mL $^{-1}$. Regarding MRSA, propolis from two regions (San Clemente 4 and San Javier 5) showed a MIC of 62.5 μg mL $^{-1}$, a result similar to the present study, in which an MPF presented a MIC of 64 μg mL $^{-1}$, demonstrating potential as an antimicrobial agent.

Bueno-Silva *et al*. (2017) verified the effect of climatic seasons on the chemical composition and antimicrobial activity of Brazilian red propolis and, through one-year analysis, observed variation of MIC from 31.2 - 62.5 to 125 - 250 μg mL $^{-1}$ and MBC from 62.5 - 125 to 125 - 250 μg mL $^{-1}$ for *S. aureus* and MIC variation from 62.5 - 125 to 125 - 250 μg mL $^{-1}$ and MBC from 250 - 500 to 500 - 1000 μg mL $^{-1}$ for *Streptococcus mutans*. A similar study conducted by Nascimento *et al*. (2019) determined an antimicrobial activity of ethanolic extracts of red propolis from different apiaries in the state of Alagoas, from March 2011 to February 2012 and verified the inhibition of growth of *S. aureus* (MIC between 118 to 500 μg mL $^{-1}$) in most of the months studied, except for a few months that coincided with the decrease in flavonoids/isoflavonoids and guttiferones present in red propolis. In a study by Regueira-Neto *et al*. (2017) in order to determine the antibacterial activity of Brazilian red propolis against clinical isolates of human origin, MIC values ranging from 53 μg mL $^{-1}$ to 512 μg mL $^{-1}$ were found against strains of *S. aureus*, and from 384 μg mL $^{-1}$ to ≥ 1024 μg mL $^{-1}$ against *Escherichia coli*. Other studies have also demonstrated *in vitro* antibacterial activity of propolis against *S. aureus*, with MICs of 56.75 μg mL $^{-1}$ (Rufatto *et al*., 2018) and 62.5-1000 μg mL^{-1} (Dantas Silva *et al*., 2017). Recent studies have indicated the components Isoliquiritigenin, vestitol, neovestitol, medicarpin and synergism of phenolic compounds are responsible for the antimicrobial properties of red propolis (Inui *et al*., 2014; Bueno-Silva *et al*., 2017).

However, the mechanism of propolis antimicrobial activity is still complex and can be attributed to the presence of several bioactive compounds, particularly isoflavonoids and a combination of them. Thus causing damage to the cytoplasmic membrane (promoted by low fluidity of the membrane), inhibition of chemical synthesis (inhibition of topoisomerase), inhibition of energy metabolism (triggered by NADH cytochrome inhibited by C reductase) or inhibition of the indication and formation of biofilms, as previously reported (Xie *et al*., 2015). Regarding the chemical composition of Brazilian red propolis, Inui *et al*. (2014) identified the phenolic compounds and evaluated the antimicrobial activity of (3S)-vestitol, (3S)-novestitol, isoliquiritigenin, (6aS,11aS)-medicarpin on bacterial isolates. These constituents exhibited antibacterial activity against *S. aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*, with low MIC values (64 mg mL $^{-1}$). In particular, the compound medicarpin exhibited better activity against *S. aureus*, *B. subtilis* and *P. aeruginosa*, with MIC values of 16, 32 and 32 mg mL $^{-1}$, respectively. Other components of propolis have also been tested, but have shown no antibacterial activity, or confirm these four compounds as determinants for antimicrobial action. In a study by Gaur *et al*. (2016), to explore the combined *in vitro* and *in vivo* effect of liquiritigenin and isolated with β-lactam antibiotics against MRSA strains, it was possible to observe what these components exhibited anti-MRSA activity (50-100 μg mL $^{-1}$), besides, isoliquiritigenin was able to significantly reduce the MIC of β-lactam antibiotics by up to 16 times, while liquiritigenin reduced by up to 8 times. Based on the results presented, it is also evident that the solvent used to obtain the extract may infer in the antimicrobial activity of red propolis. In a study proposed by Cabral *et al*. (2009), it was found that the ethanolic extract of propolis had a chemical profile similar to its chloroform fraction, however, very distinct from the hexanic fraction, to which the latter presented only a single peak and apolar nature, although not identified, according to chromatographic analysis. Analysis of phenolic compounds in this same study indicated that the ethanolic extract contained a phenolic compound content of 257 mg (AG/g), compared to 154 mg (AG/g) of the hexanic fraction and 249.75 mg (AG/g) of the chloroformic fraction. Despite this, the best results of MIC and MBC observed for *S. aureus* were for the chloroform fraction and its subfractions with mean values of 26.4 - 52.2 μg mL $^{-1}$ and 57.4 - 114.6 μg mL $^{-1}$, respectively.

Similar results were observed in a study by Bispo Junior *et al*. (2012), in which the chloroform fraction inhibited 92% of the species used in the study against 77% of the hexanic fraction. This suggests that the ideal solvent for testing propolis samples of this type will drag as many active constituents as possible responsible for its antimicrobial activity. On the other hand, the use of low polarity solvents can lead to constituents of no pharmacological interest, such as waxes, which are long chain organic compounds. Despite its great chemical variability, it is suggested that the antibacterial effect of propolis is related to

some classes of phenolic compounds of various polarities, present in different fractions of the extract and not by the synergistic effect of the fraction obtained by the raw extract (Alencar *et al*., 2007; Cabral *et al*., 2009).

Conclusion

A high incidence of environmental contamination by *S. aureus* was observed in the ICU, mainly in the filters of the air conditioner and on the surface of the entrance door. It represents a risk for the health of patients, highlighting the need for preventive interventions and programs for re-education and encouragement of good practices within the hospital unit. The results also indicated that MRSA strains can remain viable within the ICU environment being a source of contamination for patients. It is important to note that microbiological samples of air conditioners and surfaces can be useful in the investigation of important microorganisms related to HAIs. The hydroalcoholic extract, as well as the medium and medium-high polarity fractions were effective against staphylococcal strains. The fraction of medium polarity had low MIC values, most likely due to the greater amount of bioactive compounds dragged by this fraction responsible for the antimicrobial activity. Thus, products based on red propolis can be an efficient alternative for the control of pathogenic microorganisms of clinical interest.

REFERENCES

Alencar, S. M., Oldoni, T. L. C., Castro, M. L., Cabral, I. S. R., Costa-Neto, C. M., Cury, J. A., Rosalen, P. L., &Ikegaki, M. 2007. Chemical composition and biological activity of a new type of Brazilian propolis: Red propolis. *Journal of Ethnopharmacology*, 1132, 278–283. Doi: 10.1016/j.jep.2007.06.005.

Alvarez, C., Labarca, J., & Salles, M. 2010. Prevention strategies for methicillin-resistant *Staphylococcus aureus* MRSA in Latin America. *Brazilian Journal of Infectious Diseases*, 142, 107-118. Doi: 10.1590/S1413-86702010000800006.

Andrade, D., Leopoldo, V. C., & Haas, V. J. 2006. Occurrence of multi-resistant bacteria in the intensive care unit of a Brazilian hospital of emergencies. *Revista Brasileira de Terapia Intensiva*, 181, 27-33. Doi: 10.1590/S0103-507X2006000100006.

Bispo Júnior, W., Miranda, E. O., Alvino, V., Araújo, B., Silva, D. W., & Porfirio, Z. 2012. Antimicrobial activity of fractions of red propolis from Alagoas, Brazil. *Semina: Ciências Biológicas e da Saúde*, 331, 03-10. Doi: 10.5433/1679-0367.2012v33n1p03.

Boretti, V. S., Corrêa, R. N., Santos, S. S., Leão, M. V., & Gonçalves e Silva, C. R. 2014. Sensitivity profile of *Staphylococcus* spp. and *Streptococcus* spp. isolated from toys used in a teaching hospital playroom. *Revista paulista de pediatria*, 323, 151–156. Doi: 10.1590/0103-0582201432301.

Bride, L. L., Pereira, M. F., Barbosa, M. C., Silva, N. C., Klein, N. M., Nascimento, T. C., &Schuenck, R. P. 2019. Differences in resistance profiles and virulence genes among methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* of different lineages at a public tertiary hospital. *Revista da Sociedade Brasileira de Medicina Tropical*, 52, 2-7. Doi: 10.1590/0037-8682-0095-2019.

Bueno-Silva, B., Alencar, S. M., Koo, H., Ikegaki, M., Silva, G. V., Napimoga, M. H., &Rosalen, P. L. 2013. Anti-Inflammatory and antimicrobial evaluation of neovestitol and vestitol isolated from Brazilian Red Propolis. *Journal of Agricultural and Food Chemistry*, 6119, 4546-4550. Doi: 10.1021/jf305468f.

Bueno-Silva, B., Koo, H., Falsetta, M. L., Alencar, S. M., Ikegaki, M., &Rosalen, P. L. 2013. Effect of neovestitol–vestitol containing Brazilian red propolis on accumulation of biofilm *in vitro* and development of dental caries *in vivo*. *Biofouling*, 2910, 1233–1242. Doi: 10.1080/08927014.2013.834050.

Bueno-Silva, B., Marsola, A., Ikegaki, M., Alencar, S. M., &Rosalen, P. L. 2017. The effect of seasons on Brazilian red propolis and its botanical source: Chemical composition and antibacterial activity. *Natural Product Research*, 3111, 1318–1324. Doi: 10.1080/14786419.2016.1239088.

Cabral, I. S. R., Oldoni, T. L. C., Prado, A., Bezerra, R. M. N., & Alencar, S. M. 2009. Phenolic composition, antibacterial and antioxidant activities of Brazilian red propolis. *Química nova*, 326, 1523-1527. Doi: 10.1590/S0100-40422009000600031.

Cabrera, R., Fernández-Barat, L., Motos, A., López-Aladid, R., Vázquez, N., Panigada, M., Álvarez-Lerma, F., López, Y., Muñoz, L., Castro, P., Vila, J., & Torres, A. 2020. Molecular characterization of methicillin-resistant *Staphylococcus aureus* clinical strains from the endotracheal tubes of patients with nosocomial pneumonia. *Antimicrobial resistance and infection control*, 91, 43. Doi: 10.1186/s13756-020-0679-z.

Carling, P. C., &Bartley, J. M. 2010. Evaluating hygienic cleaning in health care settings: what you do not know can harm your patients. *American Journal of Infection Control*, 385 Suppl 1, 41-50. Doi: 10.1016/j.ajic.2010.03.004.

Chambers, H. F., &Deleo, F. R. 2009. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. *Nature reviews. Microbiology*, 79, 629–641. Doi: 10.1038/nrmicro2200.

Chen, Y. H., Huang, K. A., Huang, Y. C., Chi, H., Lu, C. Y., Chang, L. Y., Ho, Y. H., Chi, C. Y., Liu, C. C., Huang, L. M., Yang, T., & Huang, Y. C. 2020. Prevalence and molecular characterizations of *Staphylococcus aureus* nasal colonization among patients in pediatric intensive care units in Taiwan. *Antimicrobial resistance and infection control*, 91, 41. Doi: 10.1186/s13756-020-0700-6.

Clinical and Laboratory Standards Institute CLSI 2018. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, West, Pennsylvania 19087 USA.

Clinical and Laboratory Standards Institute CLSI 2019. *Performance Standards for Antimicrobial Susceptibility Testing*. 29th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, West, Pennsylvania 19087 USA.

Dantas Silva, R. P., Machado, B. A., Barreto, G. A., Costa, S. S., Andrade, L. N., Amaral, R. G., Carvalho, A. A., Padilha, F. F., Barbosa, J. D., &Umsza-Guez, M. A. 2017. Antioxidant, antimicrobial, antiparasitic, and cytotoxic properties of various Brazilian propolis extracts. *PloS one*, 123, 1-18. Doi: 10.1371/journal.pone.0172585.

Daugsch, A., Moraes, C. S., Fort, P., & Park, Y. K. 2008. Brazilian red propolis - chemical composition and

botanical origin. *Evidence-based complementary and alternative medicine: eCAM*, *5*4, 435–441. Doi: 10.1093/ecam/nem057.

Deng, Y., Liu, J., Peters, B. M., Chen, L., Miao, J., Li, B., Li, L., Chen, D., Yu, G., Xu, Z., & Shirtliff, M. E. 2015. Antimicrobial resistance investigation on *Staphylococcus* strains in a local hospital in Guangzhou, China, 2001-2010. *Microbial DrugResistance*, 211, 102–104. Doi: 10.1089/mdr.2014.0117.

Devequi-Nunes, D., Machado, B., Barreto, G. A., Rebouças Silva, J., da Silva, D. F., da Rocha, J., Brandão, H. N., Borges, V. M., &Umsza-Guez, M. A. 2018. Chemical characterization and biological activity of six different extracts of propolis through conventional methods and supercritical extraction. *PloS one*, 1312, 1-20. Doi: 10.1371/journal.pone.0207676.

Eslami, A., Karimi, F., Karimi, Z., &Rajabi, Z. 2016. A Survey of the quantity and type of biological aerosols in selected wards of a teaching hospital in Ghazvin. *Electronicphysician*, 84, 2281–2285. Doi: 10.19082/2281.

Ferreira, A. M., Andrade, D., Rigotti, M. A., & Almeida, M. T. G. 2011. Methicillin-resistant *Staphylococcus aureus* on surfaces of an Intensive Care Unit. *Acta Paulista de Enfermagem*, 244, 453-458. Doi: 10.1590/S0103-21002011000400002.

Gaur, R., Gupta, V. K., Singh, P., Pal, A., Darokar, M. P., &Bhakuni, R. S. 2016. Drug resistance reversal potential of isoliquiritigenin and liquiritigenin isolated from *Glycyrrhiza glabra* against Methicillin-Resistant *Staphylococcus aureus* MRSA. *Phytotherapy Research*, 3010, 1708-1715. Doi: 10.1002/ptr.5677.

Gebel, J., Exner, M., French, G., Chartier, Y., Christiansen, B., Gemein, S., Goroncy-Bermes, P., Hartemann, P., Heudorf, U., Kramer, A., Maillard, J. Y., Oltmanns, P., Rotter, M., & Sonntag, H. G. 2013. The role of surface disinfection in infection prevention. *GMS hygiene and infection control*, *8*1, 1-12. Doi: 10.3205/dgkh000210.

Gomes, N. C. P., Ferreira, L. G., &Lembo, T. 2016. Análise da contaminação bacteriológica do setor de parada de ônibus municipais do terminal rodoviário de uma cidade do interior do estado de São Paulo. *Journal of the Health Sciences Institute*, 343, 140-143.

Han, J. H., Sullivan, N., Leas, B. F., Pegues, D. A., Kaczmarek, J. L., &Umscheid, C. A. 2015. Cleaning hospital room surfaces to prevent Health Care-Associated Infections: a technical brief. *Annals of internal medicine*, *163*8, 598–607. Doi: 10.7326/M15-1192.

Inui, S., Hatano, A., Yoshino, H., Hosoya, T., Shimamura, Y., Masuda, S., Ahn, M. R., Tazawa, S., Araki, Y., &Kumazawa, S. 2014. Identification of the phenolic compounds contributing to antibacterial activity in ethanol extracts of Brazilian red propolis. *Natural Product Research*, 2816, 1293-1296. Doi: 10.1080/14786419.2014.898146.

Kondo, N., Kuwahara-Arai, K., Kuroda-Murakami, H., Tateda-Suzuki, E., &Hiramatsu, K. 2001. Eagle-type methicillin resistance: new phenotype of high methicillin resistance under *mec* regulator gene control. *Antimicrobial agents and chemotherapy*, 453, 815–824. Doi: 10.1128/AAC.45.3.815-824.2001.

Kondo, Y., Ito, T., Ma, X. X., Watanabe, S., Kreiswirth, B. N., Etienne, J., &Hiramatsu, K. 2007. Combination of multiplex PCRs for staphylococcal cassette chromosome *mec* type assignment: rapid identification system for *mec*, *ccr*, and major differences in junkyard regions. *Antimicrobial agents and chemotherapy*, 511, 264–274. Doi: 10.1128/AAC.00165-06.

Kramer, A., Schwebke, I., &Kampf, G. 2006. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infectious Diseases*, 6130, 1-8. Doi: 10.1186/1471-2334-6-130.

Kumar, K. M., Anbarasu, A., & Ramaiah, S. 2014. Molecular docking and molecular dynamics studies on β-lactamases and penicillin binding proteins. *Molecular BioSystems*, 104, 891–900. Doi: 10.1039/c3mb70537d.

Libério, S. A., Pereira, A. L., Araújo, M. J., Dutra, R. P., Nascimento, F. R., Monteiro-Neto, V., Ribeiro, M. N., Gonçalves, A. G., &Guerra, R. N. 2009. The potential use of propolis as a cariostatic agent and its actions on mutans group streptococci. *Journal of Ethnopharmacology*, 1251, 1-9. Doi: 10.1016/j.jep.2009.04.047.

Libert, X., Chasseur, C., Packeu, A., Bureau, F., Roosens, N. H., & De Keersmaecker, S. 2019. Exploiting the advantages of molecular tools for the monitoring of fungal indoor air contamination: first detection of *Exophialajeanselmei* in indoor air of air-conditioned offices. *Microorganisms*, *7*12, 674. Doi: 10.3390/microorganisms7120674.

Lopez, B. G., Lourenço, C. C., Alves, D. A., Machado, D., Lancellotti, M., &Sawaya, A. C. 2015. Antimicrobial and cytotoxic activity of red propolis: an alert for its safe use. *Journal of Applied Microbiology*, 1193, 677-87. Doi: 10.1111/jam.12874.

Lustosa, S. R., Galindo, A. B., Nunes, L. C. C., Randau, K. P., &Rolim Neto, P. J. 2008. Própolis: atualizações sobre a química e a farmacologia. *Revista Brasileira de Farmacognosia*, 183, 447-454. Doi: 10.1590/S0102-695X2008000300020.

Machado, C. S., Mokochinski, J. B., de Lira, T. O., de Oliveira, F., Cardoso, M. V., Ferreira, R. G., Sawaya, A. C., Ferreira, A. G., Pessoa, C., Cuesta-Rubio, O., Monteiro, M. C., de Campos, M. S., & Torres, Y. R. 2016. Comparative study of chemical composition and biological activity of yellow, green, brown, and red Brazilian propolis. *Evidence-based complementary and alternative medicine: eCAM*, 2016, 1-11. Doi: 10.1155/2016/6057650.

MacVane, S. H. 2017. Antimicrobial resistance in the intensive care unit: a focus on Gram-negative bacterial infections. *Journal of Intensive Care Medicine*, *32*1, 25–37. Doi: 10.1177/0885066615619895.

Mehta, Y., Hegde, A., Pande, R., Zirpe, K. G., Gupta, V., Ahdal, J., Qamra, A., Motlekar, S., & Jain, R. 2020. Methicillin-resistant *Staphylococcus aureus* in intensive care unit setting of India: a review of clinical burden, patterns of prevalence, preventive measures, and future strategies. *Indianjournal of criticalcare medicine*, 241, 55–62. Doi: 10.5005/jp-journals-10071-23337.

Miranda, S. L. F., Damasceno, J. T., Faveri, M., Figueiredo, L., da Silva, H. D., Alencar, S. M., Rosalen, P. L., Feres, M., &Bueno-Silva, B. 2019. Brazilian red propolis reduces orange-complex periodontopathogens growing in multispecies biofilms. *Biofouling*, 353, 308-319. Doi: 10.1080/08927014.2019.1598976.

Mobin, M., &Salmito, M. A. 2006. Fungus microbiota in air conditioners in intensive care units in Teresina, Piauí. *Revista da Sociedade Brasileira de Medicina Tropical*, 396, 556-559. Doi: 10.1590/S0037-86822006000600009.

Monzote, L., Cuesta-Rubio, O., Fernandez, M. C., Hernandez, I. M., Fraga, J., Pérez, K., Kerstens, M., Maes, L., & Cos,

P. 2012. *In vitro*antimicrobial assessment of Cubanpropolisextracts. Memórias do Instituto Oswaldo Cruz, 1078, 978-984. Doi: 10.1590/S0074-02762012000800003.

Murray, P. R., Rosenthal, K. S., &Pfaller, M. A. 2017. *Microbiologia médica*. 8ª. ed. Rio de Janeiro: Elsevier.

Nascimento, T. G., Arruda, R. E. S., Almeida, E. T. C., Oliveira, J. M. S., Basílio-Júnior, I. D., Porto, I. C. C. M., Sabino, A. R., Tonholo, J., Gray, A., Ebel, R. E., Clements, C., Zhang, T., & Watson, D. G. 2019. Comprehensive multivariate correlations between climatic effect, metabolite-profle, antioxidant capacity and antibacterial activity of Brazilian red propolis metabolites during seasonal study. *ScientificReports*, 91, 1-16. Doi:10.1038/s41598-019-54591-3.

Nina, N., Quispe, C., Jiménez-Aspee, F., Theoduloz, C., Feresín, G. E., Lima, B., Leiva, E., &Schmeda-Hirschmann, G. 2015. Antibacterial activity, antioxidant effect and chemical composition of propolis from the región del Maule, Central Chile. Molecules, 2010, 18144–18167. Doi: 10.3390/molecules201018144.

Nunes, Z. G. 2005. *Estudo da qualidade microbiológica do ar de ambientes internos climatizados*. Dissertação Mestrado em Vigilância Sanitária - Instituto de Controle da Qualidade em Saúde, Fundação Oswaldo Cruz, Rio de Janeiro - RJ.

Oliveira, A. C., & Damasceno, Q. S. 2010. Surfaces of the hospital environment as possible deposits of resistant bacteria: a review. *Revista da Escola de Enfermagem da USP*, 444, 1112-1117. Doi: 10.1590/S0080-62342010000400038.

Oliveira, A. C., & Silva, R. S. 2008. Desafios do cuidar em saúde frente à resistência bacteriana: uma revisão. *Revista Eletrônica de Enfermagem*, 101, 189-197.

Ortíz-Gil, M. Á., Velazquez-Meza, M. E., Echániz-Aviles, G., Mora-Domínguez, J. P., Carnalla-Barajas, M. N., &Mendiola Del Moral, E. L. 2020. Tracking methicillin-resistant *Staphylococcus aureus* clones in a hospital in Southern Mexico. *Saludpública de México*, 622, 186-191. Doi: 10.21149/10786.

Padoveze, M. C., & Fortaleza, C. M. C. B. 2014. Healthcare-associated infections: challenges to public health in Brazil. Revista de Saúde Pública, 48 6, 995-1001. Doi: 10.1590/S0034-8910.2014048004825.

Pereira, D. S., Freitas, C. I. A., Freitas, M. O., Maracajá, P. B., Silva, J. B. A., Silva, R. A., & Silveira, D. C. 2015. Histórico e principais usos da própolis apícola. *Agropecuária Científica no Semi-Árido*, 112, 01-21.

Pippi, B., Lana, A. J. D., Moraes, R. C., Güez, C. M., Machado, M., Oliveira, L. F. S., Von Poser, G. L., &Fuentefria, A. M. 2015. *In vitro* evaluation of the acquisition of resistance, antifungal activity and synergism of Brazilian red propolis with antifungal drugs on *Candida* spp. *Journal of Applied Microbiology*, 1184, 839-850. Doi: 10.1111/jam.12746.

Procop, G. W., Church, D. L., Hall, G. S., Janda, W. M., Koneman, E. W., Schreckenberger, P. C., & Woods, G. L. 2018. *Diagnóstico microbiológico: texto e atlas colorido*. 7ªed. Rio de Janeiro: Guanabara Koogan.

Quadros, M. E., Lisboa, H. M., Oliveira, V. L., &Schirmer, W. N. 2009. Qualidade do ar em ambientes internos hospitalares: estudo de caso e análise crítica dos padrões atuais. *Engenharia Sanitária e Ambiental*, 143, 431-438. Doi: 10.1590/S1413-41522009000300017.

Ramm, L., Siani, H., Wesgate, R., &Maillard, J. Y. 2015. Pathogen transfer and high variability in pathogen removal by detergent wipes. *American Journal of InfectionControl*, 437, 724–728. Doi: 10.1016/j.ajic.2015.03.024.

Regueira-Neto, M. S., Tintino, S. R., Silva, A. R. P., Costa, M. D. S., Boligon, A. A., Matias, E. F. F., Queiroz Balbino, V., Menezes, I. R. A., Melo Coutinho, H. D. 2017. Seasonal variation of Brazilian red propolis: Antibacterial activity, synergistic effect and phytochemical screening. *Food andChemicalToxicology*, 107Pt B, 572-580. Doi: 10.1016/j.fct.2017.03.052.

Regueira-Neto, M. S., Tintino, S. R., Silva, A. R. P., Costa, M. S., Oliveira-Tintino, C. D. M., Boligon, A. A., Menezes, I. R. A., Balbino, V. Q., & Coutinho, H. D. M 2019. Comparative analysis of the antibacterial activity and HPLC phytochemical screening of the Brazilian red propolis and the resin of *Dalbergia ecastaphyllum*. *Chemistry&Biodiversity*, 169, e1900344. Doi: 10.1002/cbdv.201900344.

Reis, J., Barreto, G. A., Cerqueira, J. C., Anjos, J., Andrade, L. N., Padilha, F. F., Druzian, J. I., & Machado, B. 2019. Evaluation of the antioxidant profile and cytotoxic activity of red propolis extracts from different regions of northeastern Brazil obtained by conventional and ultrasound-assisted extraction. *PloS one*, 147, 1-27. Doi: 10.1371/journal.pone.0219063.

Renner, J. D. P., & Carvalho, E. D. 2013. Microorganisms isolated from environmental surfaces of an adult ICU in a hospital in Vale of the Rio Pardo – RS. *Revista de Epidemiologia e Controle de Infecção*, 32, 40-44. Doi: 10.17058/reci.v3i2.3290.

Rufatto, L. C., Luchtenberg, P., Garcia, C., Thomassigny, C., Bouttier, S., Henriques, J. A. P., Roesch-Ely, M., Dumas, F., & Moura, S. 2018. Brazilian red propolis: Chemical composition and antibacterial activity determined using bioguided fractionation. *Microbiological Research*, 214, 74-82. Doi: 10.1016/j.micres.2018.05.003.

Russotto, V., Cortegiani, A., Raineri, S. M., &Giarratano, A. 2015. Bacterial contamination of inanimate surfaces and equipment in the intensive care unit. *Journal of IntensiveCare*, 354, 1-8. Doi: 10.1186/s40560-015-0120-5.

Santos Junior, A. G., Ferreira, A. M., Rigotti, M. A., Santos, F. R., Furlan, M. C. R., &Andrade, D. 2018 Avaliação da eficiência da limpeza e desinfecção de superfícies em uma Unidade Básica de Saúde. *Texto & Contexto Enfermagem*, 274, 1-10. Doi: 10.1590/0104-07072018003720017.

Santurio, J. M., Santurio, D. F., Pozzatti, P., Moraes, C., Franchin, P. R., & Alves, S. H. 2007. Antimicrobial activity of essential oils from oregano, thyme and cinnamon against *Salmonella enterica*sorovars from avian source. *Ciência Rural*, 373, 803-808. Doi: 10.1590/S0103-84782007000300031.

Sattar, S. A., Bradley, C., Kibbee, R., Wesgate, R., Wilkinson, M. A. C., Sharpe, T., & Maillard, J. Y. 2015. Disinfectant wipes are appropriate to control microbial bioburden from surfaces: Use of a new ASTM standard test protocol to demonstrate efficacy. *Journal of Hospital Infection*. 914, 319–325. Doi: 10.1016/j.jhin.2015.08.026.

Siboo, I. R., Cheung, A. L., Bayer, A. S., &Sullam, P. M. 2001. Clumping factor A mediates binding of *Staphylococcus aureus* to human platelets. *Infectionandimmunity*, 695, 3120–3127. Doi: 10.1128/IAI.69.5.3120-3127.2001.

Silva, D. P., Nazaré, D. L., Muniz, J. W. C., & Câmara, C. N. S. 2013. Hospital-acquired infections associated with poor

air quality in air-conditioned environments. *Revista de Epidemiologia e Controle de Infecção*, 34, 153-157.

Silva, F. R. G., Matias, T. M. S., Souza, L. I. O., Matos-Rocha, T. J., Fonseca, S. A., Mousinho, K. C., & Santos, A. F. 2019. Phytochemical screening and *in vitro* antibacterial, antifungal, antioxidant and antitumor activities of the red propolis Alagoas. *Brazilian Journal of Biology*, 793, 452-459. Doi: 10.1590/1519-6984.182959.

Silva-Beltrán, N. P., Balderrama-Carmona, A. P., Umsza-Guez, M. A., &Souza Machado, B. A. 2019. Antiviral effects of Brazilian green and red propolis extracts on Enterovirus surrogates. *Environmental Science and Pollution Research*. Doi: 10.1007/s11356-019-07458-z.

Streit, J. M., Jones, R. N., Sader, H. S., &Fritsche, T. R. 2004. Assessment of pathogen occurrences and resistance profiles among infected patients in the intensive care unit: report from the SENTRY Antimicrobial Surveillance Program North America, 2001. *International Journal of Antimicrobial Agents*, 242, 111-118. Doi: 10.1016/j.ijantimicag.2003.12.019.

Vickery, K., Deva, A., Jacombs, A., Allan, J., Valente, P., &Gosbell, I. B. 2012 Presence of biofilm containing viable multiresistant organisms despite terminal cleaning on clinical surfaces in an intensive care unit. *Journal of Hospital Infection*, 801, 52–55. Doi: 10.1016/ j.jhin.2011.07.007.

Weber, D. J., Anderson, D., &Rutala, W. A. 2013. The role of the surface environment in healthcare-associated infections. *Current Opinion in Infectious Diseases*, 264, 338-344. Doi: 10.1097/QCO.0b013e3283630f04.

Xie, Y., Yang, W., Tang, F., Chen, X., & Ren, L. 2015. Antibacterial activities of flavonoids: structure-activity relationship and mechanism. *Current Medicinal Chemistry*, 221, 132-149. Doi: 10.2174/092986732166614 0916113443.

Zilahi, G., Artigas, A., & Martin-Loeches, I. 2016. What's new in multidrug-resistant pathogens in the ICU?. *Annals of intensivecare*, *6*96, 1-12. Doi: 10.1186/s13613-016-0199-4.

****