Antibacterial and antibiofilm activities of quercetin against clinical isolates of Staphylococcus aureus and Staphylococcus saprophyticus with resistance profile

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Abstract — The aim of this study was to determine the antibacterial and antibiofilm properties of quercetin against clinical isolates of Staphylococcus aureus and Staphylococcus saprophyticus with resistance profile. The antibacterial activity of quercetin was performed by the determination of the minimum inhibitory concentration (MIC) through the microdilution method according to the Clinical and Laboratory Standards Institute (CLSI). The percentage of inhibition of Staphylococcus spp. biofilm, after treatment with sub-inhibitory concentrations of quercetin (MIC/2 and MIC/4), was evaluated by the violet crystal assay. Quercetin showed an antimicrobial activity against clinical isolates of methicillin-susceptible S. aureus (MSSA) (MIC = 250 µg/ml), methicillin-resistant S. aureus (MRSA) (MIC = 500 µg/ml), vancomycin-intermediate S. aureus (VISA) (MIC = 125 and 150 µg/ml), S. saprophyticus resistant to oxacillin (MIC = 62.5 to 125 µg/ml), vancomycin-resistant S. aureus (VRSA) and S. saprophyticus resistant to oxacillin and vancomycin (MIC = 500 to 1000 µg/ml). At MIC/2 and MIC/4 the quercetin inhibit 46.5 ± 2.7% and 39.4 ± 4.3% of the S. aureus biofilm, respectively, and 51.7 ± 5.5% and 46.9 ± 5.5% of the S. saprophyticus biofilm, respectively. According to the results of this study, it was noticed that the quercetin presented an antibacterial activity against strains of Staphylococcus spp. with resistance profile and also inhibited the bacterial biofilm production even in sub-inhibitory concentrations.

Keywords— Resistance; biofilm; quercetin; antibacterial activity; antibiofilm activity.

I. INTRODUCTION

Staphylococcus aureus is one of the most important pathogens causers of infections in humans due to its prevalence in hospital and community contaminations [1]. In general, S. aureus is associated with superficial and deep infections in skin and soft tissues, as well as toxin-mediated diseases such as staphylococcal scalded skin syndrome, toxic shock syndrome and bacteremia with abscess formation that could lead, often, to the death of patient [1-3].

Resistant staphylococcal strains were observed shortly after the use of penicillin G in the medical clinic, in 1941. A few years later, in 1950, about 80% of the hospital samples of Staphylococcus were resistant to penicillin G, due to the production of penicillinases enzymes that inactivate this drug. Methicillin, oxacillin and its derivatives, as well as the first and second generation cephalosporins were used aiming to treat infections caused by Staphylococcus with resistance profile [4,5]. The resistance to these antimicrobials is increasing, mainly in hospital environments, which presents 50% of bacterial infections caused by methicillin-resistant Staphylococcus aureus (MRSA). Another alarming factor is that the resistant strains of S. aureus are widely distributed around the world [3,6-8].
**Staphylococcus saprophyticus** is also a species of the genus *Staphylococcus* that has a wide clinical importance. *S. saprophyticus* composes the normal microbiota of the skin and urinary and genital tracts. However, when there is an imbalance in the microbiota, occurs the beginning of urinary infections [2,3]. The resistance to methicillin in the *S. saprophyticus* strains has also reached a global distribution. Many studies defend that the main mechanism related to the acquisition of resistance to methicillin, in *S. saprophyticus*, is through the transfer of resistance genes present in the strains of MRSA or methicillin-resistant *S. epidermidis* [3,9].

The ability of some microorganisms to produce biofilm is another global public health concern. Biofilms are biological communities with a high degree of organization, in which microorganisms form structured, coordinated and functional communities. In addition, these biological communities are capable of produce polymeric matrices, wherein they are immersed and adhered to a biotic or abiotic surface [10,11]. Biofilm-producing microorganisms are responsible for most of the human bacterial infections, once they have colonization with greater structural stability and longevity. The biofilm promotes a protective barrier between bacteria and the environment, acting like an important virulence and pathogenicity factor, making these bacteria highly resistant to antimicrobials and host immunity [11,12]. In this way, it is important to conduct studies to identify the bacterial resistance phenotype, in order to contribute to epidemiological surveillance, especially of the genus *Staphylococcus*, one of leading causes of nosocomial infections.

The dissemination, especially in hospital environments, of these pathogens resistant to antimicrobial agents and biofilm producers, represents a serious threat to public health, implying in the therapeutic failure of many infectious diseases [13,14]. Despite of the development of new antimicrobials by pharmaceutical industry in the last three decades, infections caused by bacteria of genus *Staphylococcus* are still an alarming health problem. Therefore, it is necessary to discover new therapeutic options with antimicrobial and antibiofilm activity [13-16].

The flavonoids, secondary metabolites of the polyphenols class, are found in vegetables, fruits, nuts, honey, stems and flowers. Quercetin, 3,5,7,3′,4′-pentahydroxy flavone, is the most abundant flavonoid present in the human diet and represents about 95% of the total ingested flavonoids. This molecule is one of the most studied flavonoids due to its biological activities, such as antiviral, antimicrobial, antioxidant, antithrombotic and antitumoral. Some studies have described its antimicrobial activity against some microorganisms, such as *Bacillus subtilis, Micrococcus luteus* and *Aspergillus flavus* [17,18]. Despite of the existence of studies that already report its antimicrobial activity, there are no researches regarding its antimicrobial and antibiofilm activity against clinical isolates of *Staphylococcus* spp. resistant to vancomycin.

In this way, the aim of this study was to evaluate the antimicrobial and antibiofilm activities of quercetin against *Staphylococcus* spp. clinical isolates with resistance profile.

## II. MATERIAL AND METHODS

### 2.1 Identification of clinical isolates

*Staphylococcus* spp. clinical isolates were provided by a university hospital of Pernambuco, in the period from January to March 2017. The isolates were seeded in nutrient Agar (AN) for subsequent identification of bacteria. After that, the samples were seeded in Baird Parker Agar (BPA) base supplemented with 2% Egg yolk Tellurite emulsion (Hi-Media), incubated at 35 ± 2 °C for 48 h. The typical colonies of *S. aureus* (shiny black with an opaque ring, surrounded by a clear halo) were submitted to gram stain, catalase assay, coagulase, mannitol salt Agar assay and DNAse for *Staphylococcus aureus* identification. The colonies that did not presented typical aspects were submitted to gram stain, catalase assay and novobiocin sensitivity tests (5 µg), to identify *S. saprophyticus* (resistant to novobiocin) or *S. epidermidis* (sensitive to novobiocin) [19,20]. Methicillin-sensitive *Staphylococcus aureus* (MSSA) ATCC 29213 and MRSA ATCC 33591 were used as control strains.

### 2.2 Identification of resistance profile of the clinical isolates

The identification of resistance profile of the *Staphylococcus* spp. clinical isolates was conducted according to Clinical and Laboratory Standards Institute [21]. For the identification of MRSA, vancomycin-intermediate *Staphylococcus aureus* (VISA), vancomycin-resistant *Staphylococcus aureus* (VRSA) and *S. saprophyticus* resistant to cefoxitin, oxacillin and vancomycin were submitted to the method of disk diffusion with cefoxitin, oxacillin and vancomycin; microdilution method with oxacillin and vancomycin; as well as screening for oxacillin and vancomycin [21].

For the disk diffusion method, inocula of microorganisms were adjusted to 0.5 of the McFarland scale and seeded in Müeller Hinton Agar (MHA). Then, cefoxitin, oxacillin and vancomycin were deposited on the plates and incubated at 35 ± 2 °C for 24 h. After incubation, the inhibition halos were measured and analyzed following the CLSI cutting points [21].

The minimum inhibitory concentration (MIC) was determined by the microdilution method according to the CLSI [21]. Initially, 95 µl of Müeller Hinton Broth...
(MHB) was added to all plate wells. After, oxacillin and vancomycin were added in concentrations range from 0.5 to 256 µg/ml or 0.0625 to 32 µg/ml, respectively. Bacterial suspensions were adjusted to 0.5 of the McFarland scale, diluted and added in the wells to obtain a final concentration of 2–5 x 10^8 CFU/well. Subsequently, the plates were incubated at 35 ± 2 °C for 24 h. The MIC was determined as the lowest concentration of the standard drug able to inhibit >90% of the microbial growth through spectrophotometry at 620 nm.

The minimum bactericidal concentration (MBC) was determined after the obtained results of MIC. An aliquot of the wells with no microbial growth was inoculated in MHA and the plates were incubated at 35 ± 2 °C by 20-24 h. After this period, the MBC was determined as the lowest concentration with no microbial growth. The samples were analyzed following the CLSI cutting points [21].

In the screening test, initially, plates with Mueller Hinton Agar containing 4% NaCl and 6 µg/ml of oxacillin and plates with Brain Heart Infusion Agar (BHIA) containing 4% NaCl and 6 µg/ml of vancomycin were prepared. Then, microorganism inocula were adjusted to 0.5 of the McFarland scale and seeded in the plates. Finally, the plates were incubated at 35 ± 2 °C for 24 h. The plates were carefully observed against the light and any growth after 24 h was considered resistant to oxacillin and/or vancomycin [21].

2.3 Phenotypic characterization of biofilm production
2.3.1 Congo Red Agar test
The qualitative determination of biofilm production by clinical isolates was carried out according to the method of Congo Red Agar [22]. The isolates were adjusted to 0.5 of the McFarland scale (10^8 CFU/ml) in BHIA, incubated at 35 ± 2 °C for 24 h and seeded in plates containing Congo Red Agar. Subsequently, they were incubated in aerobic environment at 35 ± 2 °C for 48 h. After this period, the colonies with blackened coloration, with dry or rough consistency, were considered as biofilm-producers. Colonies of red color, with mucous consistency, were considered as not biofilm-producers. The experiment was performed in triplicate and in 3 different days.

2.3.2 Violet crystal staining
The quantitative determination of biofilm production was performed by the method of violet crystal staining [23]. Initially, the bacterial isolates were seeded in AN and incubated at 35 ± 2 °C for 18-24 h. Inocula were incubated in Tryptone Soy Broth (TSB) with 1% glucose for 24 h. Every culture was adjusted to 0.5 of the McFarland scale (10^8 CFU/ml) in the TSB with 1% glucose and the adjusted bacterial suspension was added to 96 wells plate with flat bottom. The plates were incubated at 35 ± 2 °C for 48 h. Then, the wells content were aspirates and washed with phosphate buffer (pH 7.4). Next, 200 µl of 99% methanol was added and incubated. After 15 minutes of incubation, the content was discarded. Subsequently, a solution of 1% of violet crystal stain was added in the wells and the plates were kept at room temperature for 30 minutes. The wells content was removed and washed with phosphate buffer. A solution of 33% glacial acetic acid was added and the optical density (OD) was measured by spectrophotometry at 570 nm (Multiskan microplate photometer FC, Thermo scientific, Madrid, Spain). Wells containing only the culture medium were used as control. The strains were classified into four categories, based on the values of ODs of bacterial biofilms, in comparison with value of the ODc (optical density of the control). The strains were classified into non-adherent if OD ≤ ODc; weak biofilm producer if ODc < OD ≤ 2 × ODc; moderate biofilm producer if 2 × OD ≤ 4 × ODc < ODc; or strong biofilm producer if 4 × ODc < OD [23]. The experiment was performed in triplicate and in 3 different days.

2.4 Antimicrobial activity of quercetin
The antimicrobial activity of quercetin (Sigma-Aldrich®) was performed by the microdilution method, already described previously, according to the CLSI [21]. The range of concentration of quercetin used in this study was 2 to 1000 µg/ml. The experiment was performed in triplicate and in 3 different days.

2.5 Biofilm formation-inhibition test
The antibiofilm activity of quercetin was carried out according to Das, Yang and Ma [24]. Initially, inocula were adjusted to 0.5 of the McFarland scale (10^8 CFU/ml) in TSB with 1% glucose and diluted to obtain bacterial cells concentration of 10^3 CFU/ml. These inocula were distributed in 96 plate flat-bottom wells and incubated at 37 ± 2 °C for 24 h. Later, the wells content was removed and quercetin was added in MIC, MIC/2 and MIC/4. The plates were incubated at 35 ± 2 °C for 24 h. Then, the wells content was aspirated and the violet crystal stain method was performed, as described in section 2.3.2. The experiment was performed in triplicate and in 3 different days.

III. RESULTS AND DISCUSSION
3.1 Identification of species and phenotypic resistance profile
The identification of microorganism’s prevalence in a given region is essential for the implementation of containment measures of infections caused by these bacteria. In addition to the knowledge of the species that cause infection, the identification of the resistance profile is of great importance for infections treatment caused by
these microorganisms [14]. The prevalence of resistant bacteria of genus Staphylococcus in hospital and community infections, especially in immunosuppressed individuals, makes these bacteria important subjects in research studies [3,6].

Bacteria of the genus Staphylococcus are recognized for their ability to develop drug resistance, prolonging the patient’s treatment time and causing high morbidity and mortality rates [3-6]. One of the main bacterial resistance profiles of the genus Staphylococcus is the resistance to oxacillin [5,6], which was identified in most S. aureus strains and in all S. saprophyticus strains of the present study.

Sina et al. [25] analyzed 1904 urogenital samples and isolated, about, 80 strains of Staphylococcus spp.. Staphylococcus aureus was identified in 30% of the samples and 70% as species of coagulase-negative Staphylococcus. Among these 70%, 50% were identified as S. saprophyticus. The proportion of resistance to methicillin was 54.17% for S. aureus and 52.50% for S. saprophyticus.

Vancomycin, an antimicrobial of the glycopeptide class, is, practically, the only option of treatment for infections caused by methicillin-resistant Staphylococcus strains. Although, vancomycin is currently demonstrating inefficiency in some cases [26,27]. The arising of clinical isolates with intermediate resistance or resistant to vancomycin is one of the reasons that worries the worldwide organizations related to public health, as well as an alert to health professionals [27].

Studies indicate that the appearance of the antibiotic resistance phenotypes of VISA is related to hospitalization and persistent infection [26,27], and may arise when a single colony of bacterial cells, formed mostly by cells that do not have resistance to vancomycin (MIC ≤ 2 µg/ml), has an antibiotic-resistant subpopulation at intermediate level (MIC = 4 to 8 µg/ml) [26]. The first cases of vancomycin resistance were only described in the year of 2000, in Rio de Janeiro and 2002 in Japan [28].

Almeida et al. [28] analyzed S. aureus clinical isolates from infections in patients of a university hospital in the city of Londrina, from 2001 to 2004, where 70% of the strains were resistant to oxacillin and none of them showed resistance to vancomycin. Moreira et al. [29] performed phenotypic tests in samples of Staphylococcus aureus from patients and members of the nursing team of a tertiary hospital to verify their resistance profile to oxacillin and vancomycin. In their study, 75% of the strains were MRSA and all were sensitive to vancomycin.

Tiwari and Sen [30] conducted an epidemiological study that estimated the presence of vancomycin resistance in samples of patients with S. aureus and coagulase-negative Staphylococcus, from a hospital in northern India. The group analyzed 783 strains of S. aureus, where 10 of them showed resistance to glycopeptides, 8 of these strains were resistant to vancomycin. Although this study was performed 11 years ago, the increasing incidence of Staphylococcus spp. with a resistance profile turns evident the worrying in the recent years, bringing the reflection that the resistance phenotype VRSA can be as frequent as the phenotype MRSA in the present day.

Hannan et al. [31] evaluated the resistance profile of 240 clinical isolates of S. aureus, obtained from 4 tertiary hospitals in Pakistan from July to December 2014. The study showed that 215 (89%) of the S. aureus strains were sensitive to vancomycin, at concentrations ranging from 1.0 to 2.0 µg/ml, while 25 (11%) of the strains exhibited MIC > 2 µg/ml.

**Table 1: Identification of the resistance phenotypic profile of Staphylococcus aureus clinical isolates.**

| Sample identification | Inhibition halos (mm) | MIC (µg/ml) | Screening | Resistance profile |
|-----------------------|-----------------------|-------------|-----------|--------------------|
|                       | OXA                   | CFO| VAN | OXA | VAN | OXA | VAN | MSA | MSSA | MRSA | VRSA |
| MSSA ATCC 29213       | 20.2 ± 1.7            | 29.5 ± 1.3 | 18.2 ± 0.6 | 1 | 2 | - | - | MSA | MSSA | MRSA | VRSA |
| LMB 150               | 18.8 ± 1.1            | 21.4 ± 1.2 | 17.7 ± 1.2 | 1 | 2 | - | - | MSSA | MRSA | MRSA | VRSA |
| LMB 151               | 16.2 ± 0.9            | 17.2 ± 1.1 | 21.1 ± 1.6 | 1 | 1 | - | - | MSSA | MRSA | MRSA | VRSA |
| LMB 152               | 20.2 ± 2.1            | 30.2 ± 1.6 | 21.3 ± 0.5 | 2 | 1 | - | - | MSSA | MRSA | MRSA | VRSA |
| MRSA ATCC 33591       | 0                     | 12.5 ± 1.2 | 25.2 ± 0.6 | > 256 | 1 | + | - | MRSA | MRSA | VRSA | VRSA |
| LMB 153               | 0                     | 14.3 ± 1.7 | 22.4 ± 1.3 | 8 | 2 | + | - | MRSA | MRSA | VRSA | VRSA |
| LMB 154               | 0                     | 11.1 ± 0.6 | 23.6 ± 1.7 | > 256 | 2 | + | - | MRSA | MRSA | VRSA | VRSA |
| LMB 155               | 0                     | 17.7 ± 1.5 | 11.4 ± 0.2 | 16 | 8 | - | - | MRSA | MRSA | VRSA | VRSA |
| LMB 156               | 0                     | 19.3 ± 0.6 | 11.8 ± 0.6 | 16 | 4 | + | - | VISA | MRSA | VRSA | VRSA |
| LMB 157               | 0                     | 18.6 ± 0.6 | 10.4 ± 0.2 | > 256 | 16 | + | + | VRSA | MRSA | VRSA | VRSA |
| LMB 158               | 0                     | 20.1 ± 0.8 | 0 | > 256 | > 32 | + | + | VRSA | MRSA | VRSA | VRSA |
**Table 2:** Identification of the resistance phenotypic profile of *Staphylococcus saprophyticus* clinical isolates.

| Sample identification | Inhibition halos (mm) | MIC (µg/ml) | Screening | Resistance profile |
|-----------------------|-----------------------|-------------|-----------|--------------------|
|                       | OXA                  | CFO         | VAN       | OXA                | VAN       |                      |
| LMB 163               | 17.3 ± 1.5           | 24.0 ± 2.3  | 8         | > 256             | 2         | +                    | -                    |
|                       |                      |             |           |                    |           |                      | *S. saprophyticus* resistant to OXA and CFO |
| LMB 164               | 0                    | 21.0 ± 1.4  | 0         | > 256             | 4         | +                    | -                    |
|                       |                      |             |           |                    |           |                      | *S. saprophyticus* resistant to OXA and CFO |
| LMB 165               | 0                    | 21.0 ± 1.3  | 0         | 32                 | 2         | +                    | -                    |
|                       |                      |             |           |                    |           |                      | *S. saprophyticus* resistant to OXA and CFO |
| LMB 166               | 8.2 ± 0.9            | 12.1 ± 0.7  | 2         | > 256             | > 32      | +                    | +                    |
|                       |                      |             |           |                    |           |                      | *S. saprophyticus* resistant to OXA, CFO and VAN |
| LMB 167               | 0                    | 17.4 ± 2.1  | 0         | > 256             | > 32      | +                    | +                    |
|                       |                      |             |           |                    |           |                      | *S. saprophyticus* resistant to OXA, CFO and VAN |
| LMB 168               | 0                    | 18.3 ± 1.1  | 0         | > 256             | > 32      | +                    | +                    |
|                       |                      |             |           |                    |           |                      | *S. saprophyticus* resistant to OXA, CFO and VAN |
| LMB 169               | 0                    | 12.8 ± 0.7  | 0         | > 256             | > 32      | +                    | +                    |
|                       |                      |             |           |                    |           |                      | *S. saprophyticus* resistant to OXA, CFO and VAN |
| LMB 170               | 0                    | 10.1 ± 0.2  | 0         | > 256             | > 32      | +                    | +                    |
|                       |                      |             |           |                    |           |                      | *S. saprophyticus* resistant to OXA, CFO and VAN |
| LMB 171               | 0                    | 0           | 0         | > 256             | 16        | +                    | +                    |
|                       |                      |             |           |                    |           |                      | *S. saprophyticus* resistant to OXA, CFO and VAN |

MSSA: methicillin-sensitive *Staphylococcus aureus*; MRSA: methicillin-resistant *Staphylococcus aureus*; VISA: vancomycin-intermediate *S. aureus*; VRSA: vancomycin-resistant *Staphylococcus aureus*; ATCC: American Type Culture Collection; MIC: Minimum Inhibitory Concentration; LMB: Laboratory of Microbiology; OXA: Oxacillin; VAN: Vancomycin.

**3.2 Phenotypic characterization of biofilm production**

In the Congo Red Agar test, all 22 *Staphylococcus* clinical isolates were characterized as biofilm-producers (fig. 1). In the violet crystal method, all strains were characterized as biofilm-producers, being 1 classified as a low producer (4.5%), 10 as strongly biofilm-producer (45.5%) and 11 as moderately biofilm-producer (50%) (Table 3). This compatibility in the results for quantitative and qualitative methods that evaluated the biofilm production by bacteria of the genus *Staphylococcus* has been described in other studies [32,33].

**Fig. 1:** Evaluation of biofilm production by Congo Red Agar test.
MSSA: Methicillin-sensitive *Staphylococcus aureus*; MRSA: Methicillin-resistant *Staphylococcus aureus*; LMB: Laboratory of Microbiology.

According to the national health institutes publications, microorganisms that produce biofilm are related to more than 65-80% of the bacterial infections [32-35]. Hassan et al. [32] evaluated the ability of biofilm production in 110 clinical isolates of pathogenic bacteria, of different species, by the method of violet crystal staining. The obtained results were similar to our results that showed production of biofilm in all strains (100%), of which, 22.7% were classified as strongly producers, 41% moderate producers and 36.3% were weak producers. Shrestha et al. [33] noticed the biofilm production in 82% of 71 clinical isolates of the genus *Staphylococcus*.

### 3.3 Antibacterial and antibiofilm activities of quercetin

In the evaluation of antimicrobial activity of quercetin against *S. aureus* and *S. saprophyticus*, with different resistance profiles, it’s observed that this molecule has a bacteriostatic effect against all microorganisms tested. Quercetin exhibit MIC values ranged from 250 to 1000 µg/ml for *Staphylococcus aureus* (Table 4) and 62.5 to 1000 µg/ml for *Staphylococcus saprophyticus* (Table 5). In addition, the molecule was able to inhibit the biofilm production by these bacteria, even when analyzed in sub-inhibitory concentrations (Tables 4 and 5).

Quercetin showed MIC of 250 µg/ml, 500 µg/ml and 125 to 250 µg/ml against MSSA, MRSA and VISA, respectively. The best inhibitory activity of quercetin was against the *S. saprophyticus* strains resistant to oxacillin and cefoxitin (MIC = 62.5 to 125 µg/ml). The lower inhibitory activity of quercetin was observed against the VRSA strains and *S. saprophyticus* resistant to vancomycin, oxacillin and cefoxitin (MIC = 500 to 1000 µg/ml).

To show a good antibacterial activity, the molecule has to present MIC < 100 µg/ml, moderate activity with MIC between 101 and 500 µg/ml, weakly active when MIC is between 501 and 1000 µg/ml, and is inactive when MIC > 1001 µg/ml [36]. So, quercetin, in general, presented moderate antibacterial activity against the clinical isolates tested, except for VRSA and *S. saprophyticus* resistant to vancomycin, oxacillin and cefoxitin, where this molecule showed a weak activity.

Studies evaluated the antimicrobial activity of quercetin against bacterial strains using the disk diffusion or Agar diffusion method. Rauha et al. [37] observed that quercetin presented antimicrobial activity at concentration of 500 µg/ml against ATCC strains of the species: *Aspergillus niger, Bacillus subtilis, Candida albicans, Escherichia coli, Micrococcus luteus, Pseudomonas aeruginosa, Saccharomyces cerevisiae, Staphylococcus aureus* and *Staphylococcus epidermidis*, determined by the disc diffusion method. Gatto et al. [17] found no antibacterial activity of this flavonoid, in the concentration of 100 µg/ml, in any of the tested bacteria (*Staphylococcus aureus, Bacillus subtilis, Listeria ivanovi, Listeria monocytogenes, Listeria serligeri, Escherichia coli, Shigella flexneri, Shigella sonnei, Salmonella enteritidis* and *Salmonella tiphymurium*).

Nítema et al. [38] evaluated the antibacterial activity of quercetin, at a concentration of 1000 µg, through Agar diffusion method, and did not observe any activity of this molecule against bacterial strains causers of gastroenteritis. Studies that use qualitative and less precise methods, such as disk diffusion and Agar diffusion, are able to identify the antibacterial activity of quercetin, but they cannot determine the minimum inhibitory concentration. Thus, quantitative methods are important for a future *in vivo* drugs application, because they help in the determination of the dose that will be used in the treatment of infection, in humans and animals [16].

### Table 3: Biofilm production from clinical isolates of the genus Staphylococcus.

| Sample identification | Bacteria identification | Congo Red Agar test | Violet crystal staining assay |
|-----------------------|-------------------------|---------------------|-----------------------------|
| MSSA ATCC 29213       | *S. aureus*             | +                   | Strong                      |
| LMB 150               | *S. aureus*             | +                   | Strong                      |
| LMB 151               | *S. aureus*             | +                   | Strong                      |
| LMB 152               | *S. aureus*             | +                   | Strong                      |
| MRSA ATCC 33591       | *S. aureus*             | +                   | Moderate                    |
| LMB 153               | *S. aureus*             | +                   | Strong                      |
| LMB 154               | *S. aureus*             | +                   | Weak                       |
| LMB 155               | *S. aureus*             | +                   | Moderate                    |
| LMB 156               | *S. aureus*             | +                   | Moderate                    |
| LMB 157               | *S. aureus*             | +                   | Strong                      |
| LMB 158               | *S. aureus*             | +                   | Moderate                    |
Table 4: Antibacterial and antifilm activities of quercetin against Staphylococcus aureus clinical isolates.

| Sample identification | Resistance profile | Biofilm production | MIC of QUER (µg/ml) | % of biofilm inhibition |
|-----------------------|-------------------|--------------------|----------------------|-------------------------|
|                       |                   |                    | MIC          | MIC/2      | MIC/4      |
| MSSA ATCC 29213       | MSSA              | Strong             | 250          | 49.4 ± 1.2 | 43.4 ± 3.1 | 34.6 ± 1.4 |
| LMB 150               | MSSA              | Strong             | 250          | 47.3 ± 0.9 | 44.7 ± 0.8 | 36.3 ± 3.9 |
| LMB 151               | MSSA              | Strong             | 250          | 49.4 ± 2.1 | 44.1 ± 2.3 | 33.1 ± 2.0 |
| LMB 152               | MSSA              | Strong             | 250          | 48.4 ± 1.7 | 45.4 ± 2.2 | 37.8 ± 1.4 |
| MRSA ATCC 33591       | MRSA              | Moderate           | 500          | 52.8 ± 0.6 | 49.7 ± 1.4 | 42.2 ± 0.9 |
| LMB 153               | MRSA              | Strong             | 500          | 48.4 ± 1.5 | 43.9 ± 3.9 | 35.5 ± 0.5 |
| LMB 154               | MRSA              | Weak               | 500          | 58.3 ± 1.4 | 52.4 ± 1.5 | 46.1 ± 1.6 |
| LMB 155               | VISA              | Moderate           | 250          | 55.3 ± 2.4 | 48.9 ± 0.6 | 41.5 ± 1.3 |
| LMB 156               | VISA              | Moderate           | 125          | 54.6 ± 2.0 | 48.6 ± 0.8 | 44.5 ± 2.3 |
| LMB 157               | VRSA              | Strong             | 1000         | 47.1 ± 1.7 | 44.7 ± 1.7 | 36.6 ± 0.9 |
| LMB 158               | VRSA              | Moderate           | 500          | 57.2 ± 1.8 | 47.4 ± 2.2 | 43.7 ± 1.1 |
| LMB 159               | VRSA              | Moderate           | 500          | 55.5 ± 0.8 | 46.6 ± 2.4 | 43.9 ± 1.8 |
| LMB 160               | VRSA              | Moderate           | 500          | 58.5 ± 2.9 | 49.5 ± 0.5 | 44.5 ± 0.8 |
| LMB 161               | VRSA              | Strong             | 1000         | 46.7 ± 0.9 | 44.7 ± 0.6 | 36.4 ± 1.7 |
| LMB 162               | VRSA              | Strong             | 1000         | 47.7 ± 1.4 | 43.8 ± 1.7 | 35.2 ± 3.1 |

MSSA: Methicillin-sensitive Staphylococcus aureus; MRSA: Methicillin-resistant Staphylococcus aureus; VISA: Vancomycin-intermediate Staphylococcus aureus; VRSA Vancomycin-resistant Staphylococcus aureus; ATCC: American Type Culture Collection; LMB: Laboratory of Microbiology; MIC: Minimum Inhibitory Concentration; QUER: Quercetin.
Table 5: Antibacterial and antibiofilm activities of quercetin against S. saprophyticus clinical isolates.

| Sample identification | Resistance profile | Biofilm production | MIC of QUER (µg/ml) | % biofilm inhibition |
|-----------------------|--------------------|--------------------|---------------------|---------------------|
| LMB 163               | S. saprophyticus resistant to OXA and CFO | Moderate | 62.5 | 60.2 ± 1.4 | 55.5 ± 1.8 | 49.1 ± 1.2 |
| LMB 164               | S. saprophyticus resistant to OXA and CFO | Moderate | 125 | 59.6 ± 0.9 | 54.8 ± 0.6 | 48.4 ± 1.3 |
| LMB 165               | S. saprophyticus resistant to OXA and CFO | Moderate | 125 | 59.7 ± 1.7 | 53.2 ± 0.8 | 47.9 ± 2.1 |
| LMB 166               | S. saprophyticus is resistant to OXA, CFO and VAN | Strong | 500 | 50.4 ± 0.6 | 43.8 ± 1.2 | 39.2 ± 1.5 |
| LMB 167               | S. saprophyticus resistant to OXA, CFO and VAN | Strong | 500 | 52.3 ± 2.4 | 45.1 ± 1.3 | 40.3 ± 0.8 |
| LMB 168               | S. saprophyticus resistant to OXA, CFO and VAN | Strong | 1000 | 50.9 ± 1.4 | 44.6 ± 0.9 | 40.6 ± 1.1 |
| LMB 169               | S. saprophyticus resistant to OXA, CFO and VAN | Moderate | 500 | 62.8 ± 0.7 | 56.4 ± 0.9 | 52.2 ± 2.3 |
| LMB 170               | S. saprophyticus resistant to OXA, CFO and VAN | Moderate | 1000 | 60.7 ± 3.1 | 56.4 ± 1.1 | 51.1 ± 0.5 |
| LMB 171               | S. saprophyticus resistant to OXA, CFO and VAN | Moderate | 1000 | 61.5 ± 0.8 | 55.9 ± 2.4 | 53.4 ± 1.3 |

LMB: Laboratory of Microbiology; MIC: Minimum Inhibitory Concentration; QUER: Quercetin; OXA: Oxacillin; VAN: Vancomycin; CFO: Cefoxitin.

Additionally, research shows the potential of quercetin combined with other drugs for bacterial infections treatment caused by *Staphylococcus* spp. Hirai et al. [39] analyzed the activity of quercetin in combination with other antimicrobials against MRSA strains. Quercetin, in the concentration of 50 µg/ml, enhanced *in vitro* antibacterial activity of ampicillin (0.5 µg/ml), erythromycin (8 µg/ml), gentamicin (0.5 µg/ml), oxacillin (0.8 µg/ml) and vancomycin (0.125 µg/ml).

Regarding the quercetin antibiofilm activity, this molecule reduces the bacterial biofilm of *S. aureus* at MIC, MIC/2 and MIC/4, when compared to the negative control (p < 0.05). Quercetin, at MIC, reduced 53.2 ± 5.0%, 59.7 ± 5.5%, 51.6 ± 0.4% and 56.5 ± 5.8% against MRSA and VRSA, *S. saprophyticus* resistant to OXA and CFO and *S. saprophyticus* resistant to OXA, CFO and VAN, respectively. At MIC/2, quercetin reduced 48.67 ± 0.61%, 45.7 ± 2.0%, 54.5 ± 1.1% and 50.5 ± 5.9% the bacterial biofilm of MRSA, VRSA, *S. saprophyticus* resistant to OXÁ and CFO; and *S. saprophyticus* resistant to OXA, CFO and VAN, respectively. At MIC/4, quercetin reduced 42.2 ± 5.3%, 40.2 ± 4.4, 45.9 ± 0.8% and 48.4 ± 6.7% the bacterial biofilm of MRSA, VRSA, *S. saprophyticus* resistant to OXA and CFO, and *S. saprophyticus* resistant to OXA, CFO and VAN, respectively. Lee et al. [40] evaluated the ability of quercetin to inhibit the formation of biofilm of *S. aureus* ATCC 6538, through the method of violet crystal staining and verified 80% of inhibition on bacterial biofilm in the concentration at 50 µg/ml.

The relevance of our results in the evaluation of the antibiofilm activity of quercetin was to prove that this molecule, even in sub-inhibitory concentrations, is able to inhibit the formation of biofilm. This is an important fact, because some commercial drugs, such as macrolides, acetylhalicillin and tertrate and erythromycin, when used at lower concentrations than the values of MIC, stimulates the formation of biofilm in *Staphylococcus* strains, inducing resistance in clinical isolates of the genus *Staphylococcus* [6,41].

IV. CONCLUSION

In this study, we showed that the *S. aureus* is the major cause of bacterial infection in genus *Staphylococcus*, followed by a high incidence of *S. saprophyticus*. In addition, there is a concern on the incidence of resistant bacterial strains among patients of this hospital in Pernambuco, evidenced by the occurrence of vancomycin-resistant strains and the high incidence of strains that are strongly biofilm producers. In this way, we emphasize the need for identification of the resistance profile of clinical isolates, as well as the ability of this isolates to produce biofilm, once that these two factors are important to bacteria survival and could explain the inefficiency of many treatments. According to our results of antimicrobial and antibiofilm activities of quercetin, we can affirm that this molecule exhibited a promising
antibacterial activity against VISA and *S. saprophyticus* strains resistant to OXA and CFO and weak activity against VRSA strains and *S. saprophyticus* resistant to OXA, CFO and VAN. Regards the antibiofilm activity, even at sub-inhibitory concentrations, quercetin inhibited, approximately, 50% of the biofilm produced by isolates of *S. aureus* and *S. saprophyticus* vancomycin-resistant and, in consequence, reduced the resistance that could be caused by the increase in bacterial biofilm formation. Finally, further studies must be conducted in order to analyze the *in vivo* antibacterial activity of quercetin in infections caused by *Staphylococcus* species.

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