Strategies to control *Staphylococcus epidermidis* biofilms

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Staphylococcus epidermidis is the staphylococci species most commonly associated with bacteremia and hospital-acquired infections and has recently arisen as the leading cause of infections related to indwelling medical devices such as vascular catheters, prosthetic joints and artificial heart valves. The prevalence of *S. epidermidis* in hospital-acquired infections is due to its ability to adhere and form biofilms on biomaterial surfaces. This feature is one of the most important virulence factors found in *S. epidermidis*. In biofilm form, bacteria are protected from antimicrobial agents and the host immune system contributing to the persistence of biofilm infections. In addition, the emergence of *S. epidermidis* resistance to conventional therapies, based in the use of traditional antibiotics, leads to the failure of the current treatments used in the combat of *S. epidermidis infections* and is becoming a major concern. These facts are stimulating the continuous search for novel agents able to eradicate *S. epidermidis* biofilm infections or that can work in synergy with the currently available antimicrobial agents. New strategies have been showing encouraging *in vitro* results in controlling *S. epidermidis* biofilms and seem to be promising alternatives to standard antibiotics usually used in the treatment of *S. epidermidis* related infections.

Keywords Nosocomial infections; *Staphylococcus epidermidis*; biofilms; antimicrobial agents; antibiotics

1. General introduction

The coagulase-negative staphylococci *Staphylococcus epidermidis* is a human skin commensal microorganism. However, this bacterium can become an opportunistic pathogen and as such is often associated with bacteremia and hospital acquired infections, particularly in patients with catheters or others medical devices [1]. The main cause of their pathogenicity is their ability to adhere and form biofilms on the surfaces of the medical devices formerly mentioned. A biofilm is an aggregate of cells adhered to living or non-living surfaces and embedded in a self-produced matrix of extracellular polymeric substances (EPS). In biofilm form, bacteria are protected from antimicrobial agents and the host immune system contributing to the persistence of biofilm infections, and can be up to 1 000 fold more resistant to antibiotics and other antimicrobial agents, than their planktonic counterparts [2-4]. The high resistance presented by *Staphylococcus epidermidis* biofilm cells to antimicrobial agents boosted the persistant demand for new control strategies against this pathogenic bacterium in this mode of growth.

In fact, new strategies to control *S. epidermidis* biofilm-mediated infections have attracted the attention of many research groups and several studies have and are being done in order to find antimicrobial agents effective against this bacterium, specifically in this mode of growth. Natural subtances with possible antimicrobial properties have raised researchers' interest and are pointed as potential alternatives to antibiotics. Some examples are farnesol and other terpene alcohols, *N*-acetylcysteine (NAC), berberine, casbane diterpene, salvipisone, etc. that have been tested as novel agents against several pathogenic microorganisms such as bacteria, fungi and also virus, and namely against *S. epidermidis*. The development of adaptative resistance to antibiotics used in common clinical practice has also increased the interest on the new generation of antibiotics such as tigecycline, daptomycin, linezolid, arylomycins, etc., which are seen as therapeutic alternatives. Furthermore, the rapid emergence of resistance has highlighted the importance of antibiotics combination or with other antimicrobial agents, aiming at the reduction of the likelihood of resistance development and the enhancement of the effects of individual antimicrobial agents by synergistic action.
Some of these strategies showed encouraging *in vitro* results in controlling *S. epidermidis* biofilms and appear to be promising alternatives to standard antibiotics usually used in the treatment of *S. epidermidis* related infections.
Due to the increased involvement of *S. epidermidis* in foreignbody-related infections, the rapid development and exhibition of multiple antibiotic resistance as well as its great propensity to lead to persistent, chronic and recorrent infections, this pathogen remains a challenge and a subject of study by several research groups and continues to receive significant attention.

2. *Staphylococcus epidermidis* biofilm control strategies

2.1. Traditional antibiotic combination

As it was already referred, a worrying current problem is the increasing bacterial resistance to traditional antibiotics commonly used in hospitals to treat coagulase-negative (CoNS) infections, which is aggravated when the cells are in biofilms. Generally, antimicrobial agents alone are ineffective against *S. epidermidis* biofilms. Rifampicin, a RNA synthesis inhibitor, is among the most effective molecules for treating biofilm-related infections [5,6]. However, this antibiotic has a high propensity for rapid development of resistance [7,8,9]. This fact makes rifampicin unfeasible as monotherapy. A solution to this problem is the combination of antimicrobial agents, avoiding the use of monotherapy. Antibiotic combination represents a therapeutic option in the treatment of *S. epidermidis* infections [10]. In fact, some authors demonstrated that, for example, the combination of rifampicin with quinolones or fusidic acid could prevent the emergence of rifampicin resistance during therapy [8,11].

Furthermore to avoid resistance, this strategy can also potentiate the effect of individual antimicrobial agents by synergic action. Gomes *et al.* tested the susceptibility of *S. epidermidis* biofilms *in vitro* to traditional antibiotics alone and in double combinations at breakpoint concentration and demonstrated that there are some combinations that can be potentially considered for therapeutic use for an efficient control of *S. epidermidis* biofilm related-infection (data not shown). Rifampicin is always present in these combinations, being rifampicin+gentamicin and rifampicin+clindamycin the most active combinations in terms of CFU reduction and with the broadest range of action (Table 1).

Table 1: reduction in log CFU of *S. epidermidis* biofilm cells after overnight exposure to traditional antibiotics alone and in double combination.

***Staphylococcus epidermidis* strain**	**Rif**	**Clind**	**Gent**	**Rif+Clind**	**Rif+Gent**
117977	2.37	1.68	0.00	2.65	2.46
132034	4.01	0.15	0.56	3.68	3.11
150271	3.15	2.20	0.14	2.73	2.49
1457	1.58	1.72	0.00	2.46	2.06
9142	2.42	0.28	0.19	2.64	3.14

Legend: Rif- rifampicin; Clind- clindamycin; Gent- gentamicin.

After having tested the effect of traditional antibiotics alone and in double combination against *S. epidermidis* biofilms, Gomes *et al.* studied the effect of the most active combinations on the expression of *icaA* and *rsbU* genes, responsible for poly-*N*-acetylglucosamine/polysaccharide intercellular adhesin (PNAG/PIA) production (crucial for biofilm formation). The results demonstrated that this combinatorial therapy can cause a lower genetic expression of the two specific genes tested, and consequently can reduce biofilm formation recidivism, relatively to rifampicin alone [12].

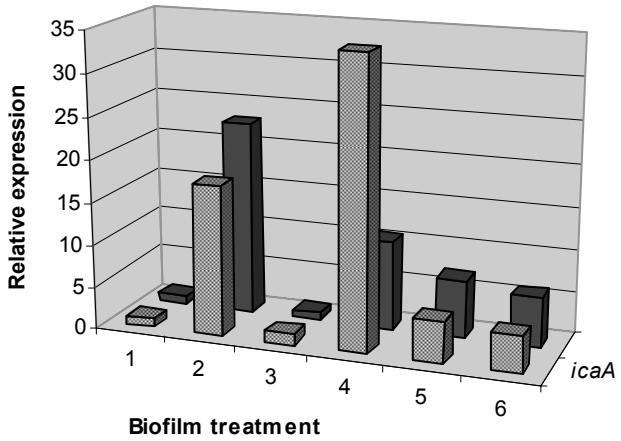

Figure 1. Relative expression of two genes responsible by PNAG/PIA formation- *icaA* and *rsbU*, in a representative *S. epidermidis* strain (*S. epidermidis* 117977) after overnight exposure to antibiotics alone and in combination: 1- positive control; 2- rifampicin; 3- clindamycin; 4- gentamicin; 5- rifampicin + clindamycin; 6- rifampicin + gentamicin.

Comparing rifampicin and both combinations tested by Gomes *et al*., it was observed that all of them have a similar effect against *S. epidermidis* biofilms (Table 1). However, taking into consideration the expression of the two genes tested, those authors concluded that biofilm cells treated with rifampicin overexpressed both genes responsible by PNAG/PIA formation, relatively to the positive control and both antibiotic combinations tested (Figure 1). This possible induction of PNAG/PIA production and consequently biofilm formation can help the bacterial cells in the evasion of the immune system. Therefore, this feature can be a possible defense and resistance mechanism adopted by *S. epidermidis* cells to protect its population from antimicrobial agents. The results of Gomes *et al.* demonstrate that, in addition to

avoid the emergence of antibiotic resistance, combinatorial therapy ensures a more efficient control of *S. epidermidis* biofilm-related infections [12].

2.2. Natural substances

The antibiotic resistance ability evidenced by microorganisms growing in biofilms boosted the continuous search for novel agents effective in the clearance of cells in this mode of growth. New *S. epidermidis* biofilm control strategies are therefore required to combat these biofilm-related infections/diseases.

Nowadays, the interest in natural substances as possible alternatives to antibiotics and new antimicrobial/anti-biofilm drugs, namely the natural substances produced by plants, whose bioactive compounds are well known for their antimicrobial properties [13], is emerging and is on the focus of some biotechnological companies. The vast range of secondary metabolites produced by plants serves to protect them against microbial pathogens and from parasitic induced damages. Perhaps it was this fact that has triggered interest in this type of compounds for the treatment of infections in humans.

2.2.1. Farnesol

Farnesol is a sesquiterpene alcohol found in the essential oils of various plant extracts including: citrus fruits [14,15], *Pluchea dioscoridis*, *Zea mays* and *Pittosporum undulatum*, possibly protecting these plants from parasitic induced damages [16,17], and can also be produced by several organisms such as the yeast *Candida albicans*. It was used during many years for therapeutic purposes, cosmetics, food flavourings, and food preservatives. Regarded as a molecule with promising antimicrobial properties, it was studied as a possible antimicrobial agent against many microorganisms and as a potential alternative to antibiotics. Derengowski *et al*. (2009) and Jabra-Rizk *et al*. (2006) were some of the authors that signaled its potential use as antimicrobial agent. Studies evidenced that farnesol affects the growth of many bacteria and fungi. One example is the coagulase-positive staphylococci *Staphylococcus aureus*, where farnesol demonstrated to inhibit biofilm formation and compromised cell membrane integrity [18,19]. Several works suggested that the main target of farnesol is the cell membrane, where the damage of this cell structure component might be one of the major modes of action of this molecule [18,19]. Exposure to terpene alcohols showed to affect cell membrane of several bacteria including *S. aureus*, *Escherichia coli*, *Listeria monocytogenes*, resulting in leakage of K^+ ions from cells [18]. Gomes and co-authors (2009) also demonstrated the antibacterial effect of farnesol against *S. epidermidis* biofilms [20]. Farnesol (300 μM) has evidenced a similar or higher effect of some traditional antibiotics [21]. For instance, it compares well with rifampicin, one of the most active antibiotics used in the treatment of *S. epidermidis*-related infections (Figure 2).

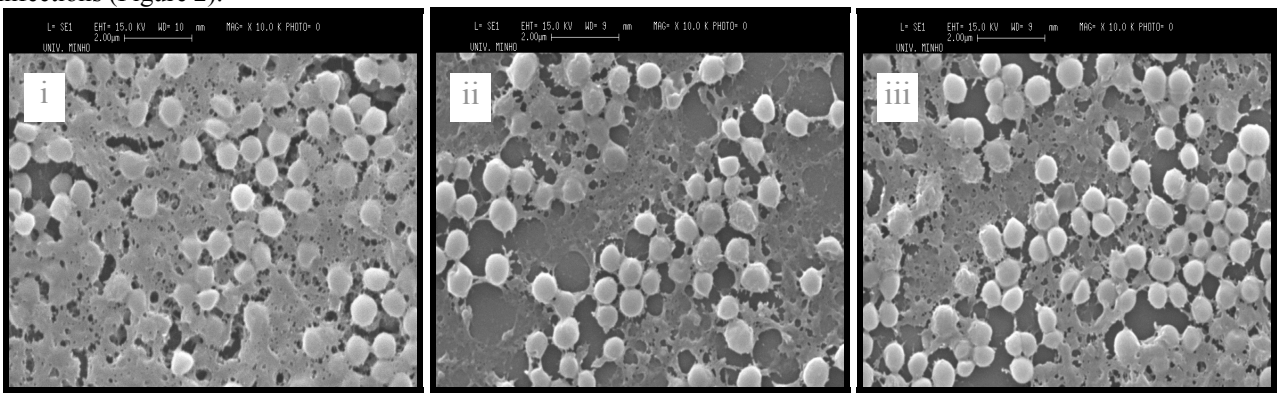

Figure 2. Scanning electron micrographs of 24 h-biofilms of *S. epidermidis* 1457 after treatment with farnesol (300 μM) or rifampicin (PS- 10 mg L^{-1}) for 24 hours. (i) non-treated cells; (ii) 300 μM farnesol; (iii) rifampicin at peak serum concentration.

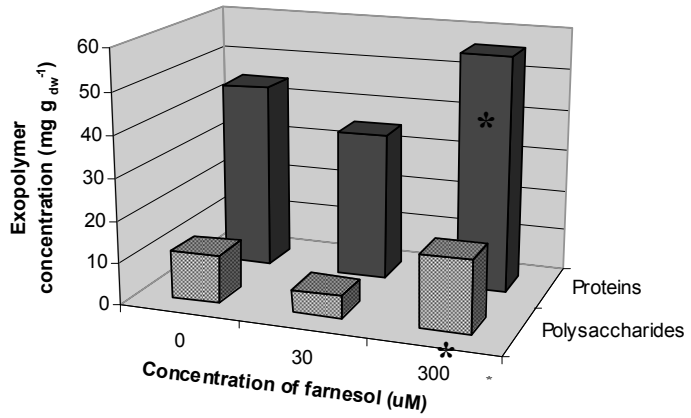

Figure 3. Concentration of exopolymers *per* gram of dry weight extracted from *S. epidermidis* 1457 biofilm matrix after 24 hours exposure to farnesol. *- concentration of exopolymers significantly different from untreated biofilm cells.

These authors demonstrated a significant increase of polysaccharide and proteins concentrations after treatment with farnesol (300 μM) (Figure 3). The justification given for this fact was the possible bursting of the cells caused by the hydrofobic nature of farnesol that favours its accumulation in the membrane, possibly causing membrane disruption and consequently the release of cellular content, as also supported by other studies [18,19].

Since *S. epidermidis* cells are able to acquire antibiotic resistance, they possibly could also develop mechanisms of resistance to farnesol. Accordingly, it was crucial to determine the cell ability to acquire tolerance/resistance to this antimicrobial agent. Subjecting consecutively *S. epidermidis* cells to sub-inhibitory and inhibitory concentrations (30 and 100 μM, respectively) of farnesol no significant reduction was observed in the antibacterial effect and susceptibility of cells to farnesol over time (Figure 4). However, it was observed a rapid adaptation of cells after the initial contact to farnesol, but since this adaptation was not progressive we concluded that this behavior is reversible, and therefore a case of tolerance and not resistance.

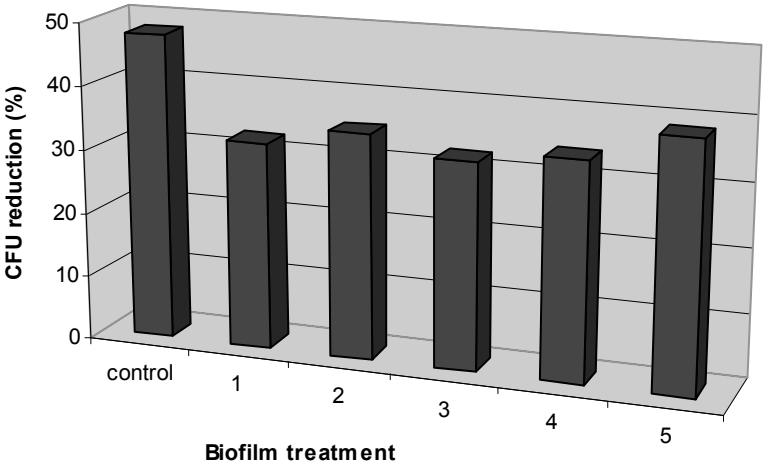

Figure 4. CFU inhibition expressed in percentage, by farnesol on *S. epidermidis* 1457 cells over five consecutive days. Control represents non-treated cells.

2.2.2. *N*-acetylcysteine (NAC)

NAC is an amino acid with strong antioxidant, antimucolytic and antibacterial properties, and is produced within the human body. *N*-Acetylcysteine decongests lungs, boosts immune response, detoxifies many toxins and poisons and boosts glutathione levels. This mucolytic substance is the smallest molecule in use (Molecular weight: 163.19) [22], being nowadays used in the treatment of chronic bronchitis [23], cancer and paracetamol intoxication [24,25]. Its effect in biofilms has been tested in several bacteria such as *Staphylocccus aureus*, *Escherichia coli* [26], *Enterobacter cloacae*, *Klebsiella pneumoniae* [27], and several other gram-positive and gram-negative bacteria [23]. In addition to reducing biofilm formation, and consequently reduce bacterial infections [23], several authors observed that NAC is able to reduce bacterial adhesion and release bacteria already adhered to the surfaces tested [23,24,28].

Biofilms are composed primarily of microbial cells and EPS. EPS may account for 50-90% of the total organic carbon of biofilms and can be considered the primary matrix material of the biofilm [29]. Since extracellular polyssacharides are the main compounds in biofilm matrices, namely in *S. epidermidis*, antimicrobial substances able to disrupt or inhibit EPS are of major interest. Taking this into consideration and knowing that NAC has mucus-dissolving properties, the effect of NAC in biofilm cells of *S. epidermidis* was also assessed [30]. In addition, and with the objective of trying to find any synergistic action between non-antibiotic substances with different modes of action and targeting different biofilm components, the combinatorial effect of NAC and farnesol was also tested (Figure 5). NAC *per se* at a concentration of 10xMIC (40 mg L^{-1}) exhibited a high inhibitory effect against *S. epidermidis* biofilm cells (Figure 5a and b), achieving reductions in the number of viable cells of *circa* of 4 log [30]. In figure 5b it is possible to observe the effect of NAC on the biofilm matrix (desintegration of biofilm matrix) and biofilm cell viability. Although farnesol and NAC act differently, no synergy was observed when tested together (Figure 5a). As observed by other researchers, NAC decreased biofilm formation and reduced the formation of extracellular polysaccharide matrix, while promoting the disruption of mature biofilm [31]. NAC has demonstrated not only to reduce adhesion but also to detach bacterial cells adhered to surfaces and to inhibit bacterial growth *in vitro* [23]. The possible action of NAC in the biofilm matrix can result in the release of cells either individually or in cell clusters, becoming the biofilm and loose cells more exposed and susceptible to the human immune system and to other antimicrobial agents. Nevertheless, the bactericidal effect of NAC is attained at a concentration that is probably too high (40 mg L^{-1}) to be administered safely but this does not invalidate its use as a lock solution therapy.

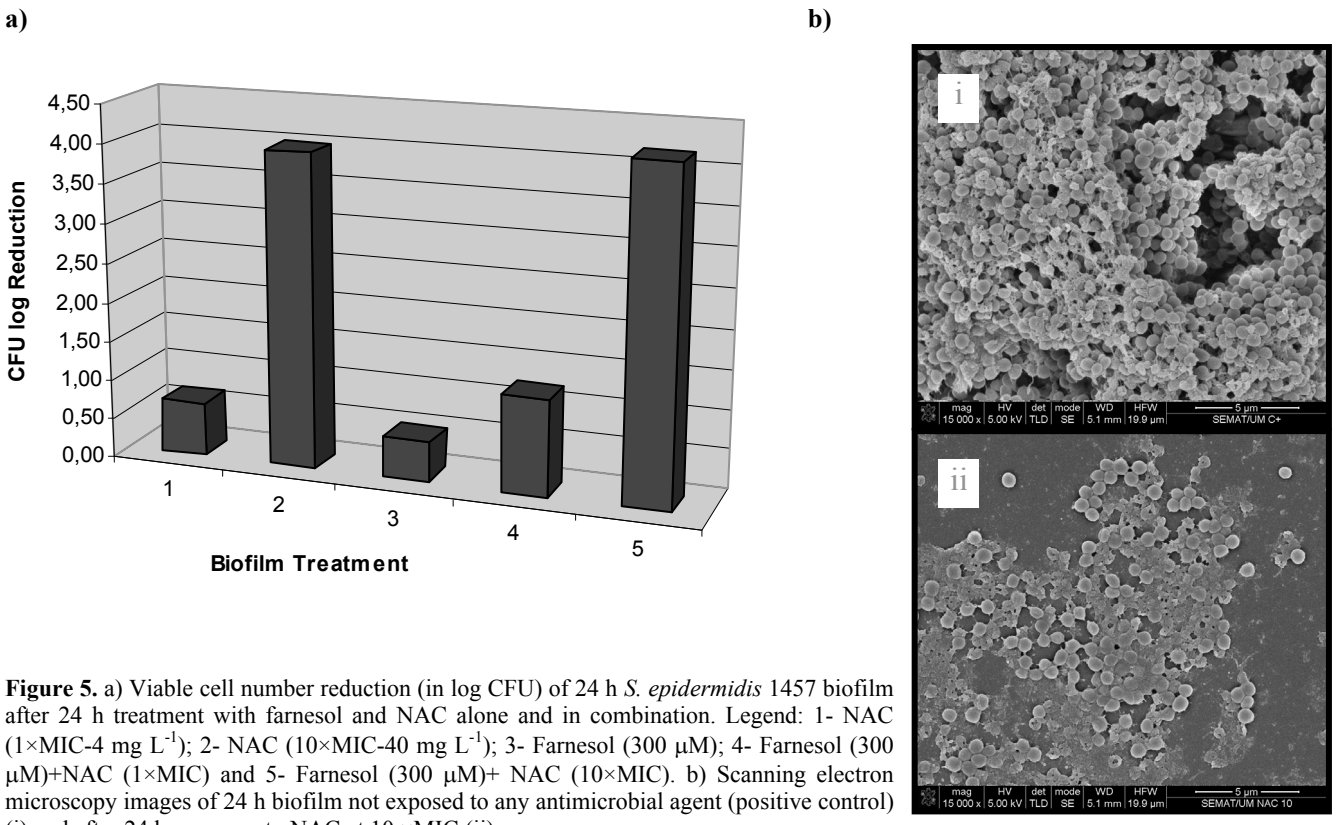

Figure 5. a) Viable cell number reduction (in log CFU) of 24 h *S. epidermidis* 1457 biofilm after 24 h treatment with farnesol and NAC alone and in combination. Legend: 1- NAC (1×MIC-4 mg L^{-1}); 2- NAC (10×MIC-40 mg L^{-1}); 3- Farnesol (300 µM); 4- Farnesol (300 µM)+NAC (1×MIC) and 5- Farnesol (300 µM)+ NAC (10×MIC). b) Scanning electron microscopy images of 24 h biofilm not exposed to any antimicrobial agent (positive control) (i) and after 24 h exposure to NAC at 10 ×MIC (ii).

2.2.3. Essential oils and other natural substances

Essential oils are described as molecules with antimicrobial activity against a wide range of microorganisms [32]. An important characteristic of essential oils and respective components is their hydrophobicity, which enables them to partition into and disturb the lipid bilayer of the cell membrane, rendering them more permeable to protons. Extensive leakage from bacterial cells or the exit of critical molecules and ions ultimately leads to bacterial cell death [33]. On the other hand, another important advantage of the usage of essential oils as alternative to antibiotics is that bacteria are not able to acquire resistance to this kind of molecules [34].

One example is cinnamon oil whose predominant active compound is cinnamaldehyde, and which is frequently used in food industry. This molecule is also described by Chang *et al*. (2001) as beneficial to human health and as having a potential use for medical purposes [35]. Nuryastuti *et al*. (2009) have shown the potential antibacterial action of this compound against *S. epidermidis* biofilm cells [36]. Cinnamon oil showed antimicrobial activity against *S. epidermidis* biofilms, translated by detachment and death of existing biofilms. The same authors, also reported a significant overexpression of the *icaA* gene, responsible by PNAG/PIA formation and therefore strongly related with biofilm formation, after exposure to sub-minimum inhibitory concentrations of cinnamon oil, pointing to a potential action of this molecule as an inducer of biofilm formation in clinical *S. epidermidis* strains. In fact, it has already been reported by several researchers that conditions potentially toxic for bacterial cells, including high levels of osmolarity, detergents, urea, ethanol, oxidative stress, and the presence of sub-MICs of some antibiotics, can be inducers of biofilm formation [37-39].

However and since cinnamon oil was able to inhibit biofilm formation, detach existing biofilms, and have a strong bactericidal effect on *S. epidermidis* biofilms, it was signaled by Nuryastuti *et al*. (2009) as an effective antimicrobial agent to combat *S. epidermidis* biofilms [36]. Based in other reports, and compared to twenty other essential oils used in traditional medicine, cinnamon oil did exhibit the most potent bactericidal effect against different important pathogens [33,40].

Berberine is an isoquinoline-type alkaloid isolated from *Coptidis rhizoma*, a major herb widely used in chinese herbal medicine [41], and in other herbs. This molecule has been pointed as a potential antimicrobial agent against a wide range of microorganisms such as bacteria, fungi and viruses. An advantage of using this molecule is its low toxicity and mutagenicity to human cells, pointing to their potential use as therapeutic alternative to traditional antibiotics. To the authors' knowledge, the only study focusing the effect of berberine on bacterial biofilms is the one by Wang *et al.* (2009) [1]. These authors defend that berberine interacts with bacterial DNA inhibiting the transcription of target genes [1]. Although the mechanism of uptake and transport of berberine in *S. epidermidis* is not fully understood, Severina *et al*. (2001) showed that this molecule penetrates phospholipid bilayers and can be

electrophoretically accumulated inside *S. aureus* cells [42]. Wang *et al*. (2009) demonstrated that berberine has a bacteriostatic effect on *S. epidermidis* cells, preventing adhesion and consequently significantly inihibiting biofilm formation [1]. These results indicate the possible use of this natural substance as an adjuvant therapeutic agent for the prevention of *S. epidermidis* biofilm-mediated infections.

Plants belonging to the genus *Salvia* have been used for years in natural medicines for treatment of several human disorders/diseases [43]. One example is the specie *Salvea sclarea* L., cultivated in many european countries, which secondary metabolites, essential oil and diterpenoids, showed various biological activities. Salvipisone is a diterpenoid isolated from *Agrobacterium rhizogenes* transformed roots of *Salvia sclarea*. Its activity against *S. epidermidis* biofilms was evidenced by the inhibition of cell adhesion and consequently the inhibition of biofilm formation. Salvipisone was shown to be bactericidal to this gram-positive pathogen, reducing significantly the number of viable biofilm cells. This compound was strongly active against *S. epidermidis* biofilms, being among the potential anti-*S. epidermidis* biofilm agents [13].

Casbane diterpene (CD) is a natural compound produced by *Croton nepetaefolius*, a native plant from northeastern Brazil [44]. This plant is widely used for medicinal purposes [45]. Testing the effect of this molecule in *S. epidermidis* biofilms, Carneiro *et al*. (2011) demonstrated that casbane diterpene affected biofilm formation by this pathogen, exhibiting an excellent antibacterial profile on gram-positive bacteria, namely on *S. epidermidis*. The possible mechanism of action, suggested by the previous research group, and supported by CD chemical characteristics, hidrophobicity and polarity, was the non-specific interaction of casbane diterpene with cell membrane [44]. The interaction with phospholipids, consequent destabilization of non-covalent interactions between the fatty acids of the lipid bilayer, possibly interfere with cellular development of *S. epidermidis* bacteria, disturbing several cellular processes such as permeability, cell growth and division.

2.3. New generation of antibiotics

Several recently available antibiotics (antibiotics of novel generation) have been developed in order to overcome the emergence of antibiotic resistance. Some examples of these new agents are: linezolid, daptomycin, tigecycline, quinupristin/dalfopristin and dalbavancin. Some authors defend that these antibiotics may be suitable for treatment of foreign-body infections, caused by sessile and biofilm-producing bacteria such as *S. epidermidis*, and may provide alternatives for monotherapy or combination therapy with rifampicin [7].

We compared the susceptibility of *S. epidermidis* biofilm cells to rifampicin, widely used in the prevention/treatment of indwelling medical device infections, with daptomycin (lipopeptide) and linezolid. Rifampicin and daptomycin showed a similar effect against *S. epidermidis* biofilms (CFU log reductions of *circa* of 3 log). Linezolid demonstrated a slightly lower activity than both antibiotics mentioned previously (Figure 6). Taking into consideration these results, we concluded that daptomycin can be strongly considered a potential alternative to rifampicin in serious infections where rifampicin resistance becomes prevalent and a possible therapeutic strategy to control *S. epidermidis*-associated infections.

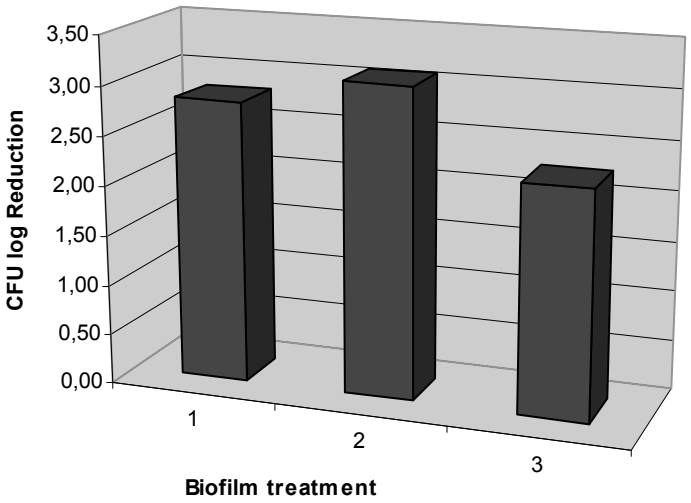

Figure 6. Viable cell number reduction (in log CFU) of 24 h *S. epidermidis* 1457 biofilm after 24 h treatment with 1- rifampicin; 2- daptomycin; 3- linezolid, at peak serum concentration (10, 95 and 18 mg L^{-1}, respectively).

Consistent with our results, other experimental data, regarding the effect of antibiotics on staphylococcal biofilms, also showed promising results for some of novel antibiotics: daptomycin, tigecycline and linezolid significantly reduced the biofilm burden and the number of viable bacteria within the biofilms [46]. Daptomycin and tigecycline (a glycylglycine) have been pointed as alternative agents to vancomycin, a standard antimicrobial agent used in the treatment of methicillin-resistant *Staphylococcus aureus* and *S. epidermidis* associated infections [47,48], presenting excellent activity against both *Staphylococci* species. Tigecycline demonstrated to be active against a range of multiresistant organisms and is bactericidal against biofilm-related *Staphylococcus epidermidis* at a lower minimal bactericidal concentration (MBC) than that of vancomycin and daptomycin [49]. Quinupristin/dalfopristin was also active against *S. epidermidis* biofilm cells [50]. *In vitro* and *in vivo* assays point to the potential usefulness of

dalbavancin for the treatment of foreign-body infections [51], caused by sessile and biofilm-producing bacteria such as *S. epidermidis*.

On the other hand, although alternative agents such as linezolid, tigecycline, daptomycin and quinupristin/dalfopristin are claimed to be highly effective against biofilms [52], and presented good results against *S. epidermidis* in the biofilm mode of growth, their use has some disadvantages and some studies presented controversial results in comparison with those mentioned previously. Firstly, apart from their very high cost, they are in clinical use for a short time only and so the extent of their toxicity is yet to be experienced [53]. Additionally, the chance to treat implant infections is very low once infection involving bacterial biofilms has begun [53]. Up to now, debridement and removal of a heavily infected implant plus antimicrobial treatment are the only options [54]. However, more experimental work has to be performed to investigate the level of debridement and the optimal type and dosage of antimicrobial substance needed for the eradication of the bacterial biofilm [44], specifically of *S. epidermidis*. Moreover, Hajdu *et al*. (2009) observed that no significant reduction in *S. epidermidis* biofilms CFU was achieved with daptomycin and tigecycline, not even at the highest concentrations tested (128 × MIC). Generally these concentrations are far beyond any concentration that can be achieved after administration of standard therapeutic doses [53]. Aslam *et al.* (2007) also tested the effect of tigecycline and after 12 hours of treatment only a mean reduction of the bacterial growth by 2 log counts was obtained, notably using a concentration of 1mg mL^{-1} (1,000 fold higher than its MIC for the organisms tested in the planktonic phase) [31]. In this case, the concentration of tigecycline expected to be in human serum after standard dosing is 2 mg L^{-1} [53]. Utilizing high doses of antimicrobials to eradicate biofilm has had limited success in the clinical practice [31]. Based on these controversial results, the use of such new antibiotics to combat infections caused by *S. epidermidis* does not seem very encouraging. In addition, resistance to some of this novel antibiotics, including linezolid [55-57], and tigecycline, also occurs, although rarely [58]. Taking this into consideration, the potential use of new antibiotics as strategies to control *S. epidermidis* infections leaves some doubt and the use of antibiotic/antimicrobial agents combinations seems to represents nowadays a very valid therapeutic option.

2.4. Antimicrobial agents combination

Considering that farnesol acts in the cells membranes, it will be expected that this compound would non-specifically potentiate the permeability of bacterial cells to certain exogenous chemical compounds, including antimicrobials, facilitating antibiotic entry and aiding in the clearance of *S. epidermidis* biofilms. This was observed by Jabra-Rizk *et al*. (2006) in a study where farnesol acted in synergy with gentamicin, pointing to a possible application of this molecule as an adjuvant therapeutic agent [18]. The interaction of farnesol with some antibiotics, such as vancomycin (cell wall synthesis inhibitor), tetracycline (protein synthesis inhibitor) and rifampicin (RNA synthesis inhibitor), with different modes of action was also studied by Gomes *et al*. However, no synergy between farnesol and the antibiotics tested was detected (data not shown) [21].

We also investigated the *in vitro* combined effect of linezolid (1 mg L^{-1}) and NAC (40 mg L^{-1}) on *S. epidermidis* biofilms. This combination exhibited a significant synergistic effect (Figure 7) and this was the most biocidal treatment herein mentioned, having promoted a 5 log reduction in the number of biofilm viable cells. For all the advantages of the combinatorial therapy above referred and the excellent inhibitory effect obtained, this combination seems to be a potential candidate in the combat of infections caused by *S. epidermidis* biofilms, at least in lock solution therapy.

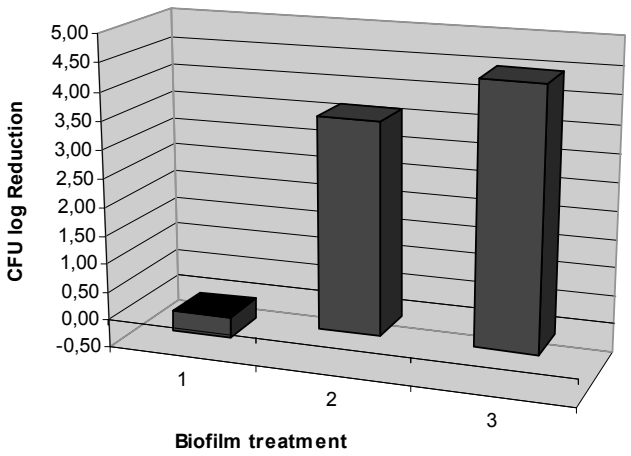

Figure 7. Viable cell number reduction (in log) of 24 h *S. epidermidis* 1457 biofilm after 24 h treatment with 1- Lin (MIC-1mg L^{-1}); 2- NAC (10×MIC-40mg L^{-1}); 3- Lin (MIC)+NAC(10×MIC).

The susceptibility of *S. epidermidis* biofilm cells to tigecycline+NAC was studied by Aslam *et al*. (2007). These authors showed that this combination significantly decreased the viable biofilm-associated bacteria and was synergistic for *S. epidermidis* [31]. A possible explanation to this increased effect may be due to the degradation of the extracellular

polysaccharide biofilm matrix by NAC [23, 26], becoming the biofilm-associated cells more susceptible to the action of tigecycline [31].

Nuryastuti *et al*. (2009) investigated the synergy between cinnamon oil and gentamicin, triclosan, and chlorhexidine on *S. epidermidis* biofilms. They showed that this essential oil has synergistic activity with all the antimicrobial agents tested. As comproved by Nuryastuti *et al*. (2009) the synergystic activity between an essential oil and an antimicrobial agent may be due to their actions on either different or similar targets of bacterial cells [36]. The synergistic activity of cinnamon oil with other antimicrobial agents could be beneficial in clinical settings, for example, to improve skin antisepsis and to eliminate antimicrobial-resistant *S. epidermidis* strains [59]. One of the advantages of the use of cinnamon oil in combinatorial therapy with a relative expensive antimicrobial agent is its low price, which can significantly lower the therapeutic cost [36].

3. Conclusions

Although new antibiotics have been developed in order to overcome the growing problem of bacteria resistance, namely of *S. epidermidis*, this does not seem to be sufficient due to the rapid emergence of resistance caused by the overuse of antibiotics, the high diversity of these bacteria, their short replication time, the horizontal transfer of resistance genes, etc. Another problem to take into consideration is the strain diversity and response to the different antimicrobial agents. The effect of antimicrobial agents is highly strain-dependent and the rate of success will be strongly dependent on the infectious *S. epidermidis* strain. The only way to avoid or slow the rapid emergence of resistance remains the prudent use of antibiotics and/or the development of alternatives to control *S. epidermidis*-related infections. Therefore it is still needed a continuous search for new strategies against *S. epidermidis* biofilms. Overall, there are some promising therapeutic strategies, such as some natural compounds and antimicrobial agents combinations, that can be possibly used for medical purposes especially in the combat of infections caused by *S. epidermidis* biofilms.

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