Revista Brasileira de ENGENHARIA BIOMÉDICA
BRAZILIAN JOURNAL OF BIOMEDICAL ENGINEERING
Volume 29, Número 2, p. 184-192, 2013

Artigo Original
http://dx.doi.org/10.4322/rbeb.2013.019

N-acetylcysteine and vancomycin alone and in combination against staphylococci biofilm

Bruna Leite, Fernanda Gomes, Poliana Melo, Clovis Souza, Pilar Teixeira, Rosário Oliveira, Elisabeth Pizzolitto*

Abstract ***Introduction:*** *The ability of staphylococci to produce biofilm is an important virulence mechanism that allows bacteria both to adhere and to live on artificial surfaces and to resist to the host immune factors and antibiotics. Staphylococcal infections have become increasingly difficult to treat due their antibiotic resistance. Therefore, there is a continuous need for new and effective treatment alternatives against staphylococcal infections. The main goal of this study was to test N-acetylcysteine (NAC) and vancomycin alone and in combination against S. epidermidis and S. aureus biofilms.* ***Methods:*** *Biofilms were treated with NAC at minimum inhibitory concentration (MIC) and 10 × MIC concentrations and vancomycin at MIC and peak serum concentrations.* ***Results:*** *The use of NAC 10 × MIC alone showed a significant antibactericidal effect, promoting a 4-5 log10 CFU/ mL reduction in biofilm cells. The combination of NAC 10 × MIC with vancomycin (independently of the concentration used) reduced significantly the number of biofilm cells for all strains evaluated (5-6 log10).* ***Conclusion:*** *N-acetylcysteine associated to vancomycin can be a potential therapeutic strategy in the treatment of infections associated to biofilms of S. epidermidis or S. aureus.*

Keywords *N-acetylcysteine, Vancomycin, Biofilm, Staphylococcus epidermidis, Staphylococcus aureus.*

*e-mail: epizzolitto@gmail.com
Recebido: 30/10/2012 / Aceito: 15/05/2013

Introduction

Staphylococci are the most frequent causative agents of nosocomial infections and infections on medical devices (Otto, 2008). Their ability to form biofilm on surfaces, either biotic or abiotic, seems to be critical for the establishment of infections and to contribute to their persistence by protecting cells from antibiotics and host defenses (Costerton *et al.*, 1999; Domínguez-Herrera *et al.*, 2012; Mah and O'Toole, 2001). *S. epidermidis* is able to form biofilms on implanted medical devices namely central venous catheter, urinary catheters, prosthetic heart valves, orthopedic devices, contact lenses, being also responsible by chronic infections such as septicemia and endocarditis (Cargill and Upton, 2009). *Staphylococcus aureus* has been recognized as a common cause of human disease (Lowy, 1998) and, as in the origin of a wide range of infections, from minor skin infections, chronic bone infections until to devastating septicemia and endocarditis (Chambers, 2005; Charles *et al.*, 2004; Davis, 2005; Gosbell, 2005; Howden *et al.,* 2004). *S. aureus* are also associated with infections related to artificial devices (Cramton and Gotz, 2004).

Biofilm formation is nowadays seen as the main virulence mechanism evidenced by staphylococci. The presence of polymeric matrix in biofilms is preponderant to bacterial adhesion and produces a complex structure that makes the eradication of microorganisms more difficult (Boles and Horswill, 2011; Kloos and Bannerman, 1994). Bacterial biofilms are difficult to detect in routine diagnostics and are inherently tolerant to host defenses and conventional antibiotic therapies (McCann *et al.*, 2008). Additionally, the emergence of antibiotic-resistant bacteria and the slow progress in identifying new classes of antimicrobial agents have encouraged research into novel therapeutic strategies (Costerton *et al.*, 2009). One of the approaches to solve this problem is the use of antimicrobial agent combinations because the development of resistance is reduced when drugs are combined (Blaser *et al.*, 1995; Chuard *et al.*, 1991; Gagnon *et al.*, 1992; Lucet *et al.*, 1990).

Moreover, combinatorial therapies are used with the aim of find a more effective alternative to antibiotics alone since combinations may allow the enhancement of the effects of individual antimicrobial agents by synergistic action.

N-acetylcysteine (NAC) is a non-antibiotic drug that has antibacterial properties (Perez-Giraldo *et al.*, 1997). NAC affects several processes that are important for bacterial biofilm formation on stainless steel surfaces, including a drastic reduction in extracellular polysaccharide production, and thus acts as an antibiofilm substance (Olofsson *et al.*, 2003). On the other hand, vancomycin is a glycopeptide that inhibit cell wall synthesis of *S. aureus* and other Gram-positive organisms (Pootoolal *et al.*, 2002). Then, the aim of this work was to determine the *in vitro* effect of two antimicrobial agents with different mode of action, alone and in combination, against biofilms of *S. epidermidis* and *S. aureus*.

Methods

Bacterial strains and growth conditions

S. epidermidis (5 strains- 1, 127, 165, 9142 and 1457) and *S. aureus* (5 strains- 2, 35, 73, 173 and 174) clinical isolate strains were used in this study. The culture media used (*tryptic soy* broth (TSB), *tryptic soy* agar (TSA) and Mueller-Hinton agar) were prepared according to the manufacturer instructions. All strains were inoculated into 15 mL of TSB from TSA plates not older than 2 days and grown for 18 (± 2) h at 37 °C in an orbital shaker at 130 rpm. Cells were harvested by centrifugation (for 5 min at 9500 *g* and 4 °C), and ressuspended in TSB adjusted to 0.5 McFarland scale equivalent to 1.5×10^8 cells/mL^{-1} before being used in the subsequent assays. Each solution of NAC (MIC and 10 × MIC) was prepared in TSB and the solution of vancomycin (MIC and peak serum (Cerca *et al.*, 2005)) was prepared in Milli-Q water.

Vancomycin MIC determination: E test

The E test was used to evaluate the susceptibility of all staphylococci strains tested to vancomycin. The cell suspension (1.5×10^8 cells/mL), prepared with *S. epidermidis* or *S. aureus,* was applied with a sterile cotton swab onto Petri plates containing Mueller-Hinton agar. The antibiotic carriers (E test strip) were then applied to each plate. Plates were incubated at 37 °C for 24 h. After incubation, an elliptical zone of growth inhibition was seen around the strip. The MIC was read from the scale at the intersection of the elliptical zone with the strip.

N-acetylcysteine MIC determination: Microbroth dilution method

For all strains of staphylococci, the *N*-acetylcysteine MIC determination was carried out in a dilution range of 0.5-64 mg/mL. The MIC was determined in 96 well tissue culture plates (Sarstedt, Newton, NC, USA) containing 100 µL of a stock solution of NAC diluted with TSB and by adding at the end 100 µL of a cell suspension (1×10^6 cells mL^{-1}). Plates were incubated at 37 °C with orbital shaking at 130 rpm for 24 h. After incubation the minimum inhibitory concentration was

Rev. Bras. Eng. Biom., v. 29, n. 2, p. 184-192, jun. 2013
Braz. J. Biom. Eng., 29(2), 184-192, June 2013

determined by identifying the lowest concentration able to inhibit bacterial growth. The controls were cells not exposed to the antimicrobial agent tested. All experiments were carried out in triplicate and repeated three times.

Congo red agar test

The Congo red agar is made up of brain heart infusion broth 37 g/L, sucrose 50 g/L, agar 10 g/L and Congo red dye 0.8 g/L. All cell suspensions (1.5×10^8 cells/mL) were seeded separately on plates containing the Congo red agar. Plates were incubated at 37 °C for 24 h. After incubation, the staphylococci that produced black colonies were considered positive slime producers, whereas the colonies that showed red color were considered negative.

Expression of gene icaD

Isolation of genomic DNA

S. epidermidis and *S. aureus* chromosomal DNA was extracted with Invitek® kit (Uniscience, Brazil).

Polymerase chain reaction-PCR

The primers used for the amplification of *icaD* genes were designed from the published sequence of the *ica* locus described by Cramton and Gotz (2004). For the detection of *icaD*, primers *iCAD*F (ATG GTC AAG CCC AGA CAG AG) and *iCAD*R (CGT GTT TTC AAC ATT TAA TGC AA) were used for a 198 bp fragment. A 20 µL reaction volume consisted of 2.5 mM MgCl2, 200 mM of each nucleotide, 1 mM of each primer, 1.25 U of Taq polymerase and 100 ng of template DNA. Thirty cycles of amplification, each consisting of denaturation at 92 °C for 45 s, annealing at 49 ºC for 45 s and elongation at 72 °C for 1 min, along with a final extension at 72 °C for 7 min were performed in a thermocycler (Eppendorf, USA). The presence and size of the amplified products were confirmed by electrophoresis on 1.5% agarose gel.

Effect of NAC and vancomycin on biofilm cells

Biofilms were formed in 96 well tissue culture plates (Sarstedt, Newton, NC, USA) containing 200 µL of a cell suspension (1×10^6 cells/mL) in TSB supplemented with 0.25 % of glucose per well to promote biofilm formation. Plates were incubated at 37 °C with orbital shaking at 130 rpm for 24 h. At the end, planktonic cells were removed carefully, and the biofilm was washed twice with 200 µL of saline solution (0.9% NaCl; Merck). The biofilms were incubated for 24 h in fresh nutrient medium containing NAC (MIC and 10 × MIC) and vancomycin (MIC and peak serum) alone and in combination.

To assess the number of viable cells after treatment, 200 µL of saline solution were added to each well before removing the biofilm by scrapping. An aliquot of 1 mL of each sample was centrifuged (for 10 min at 9500 *g* and 4 °C) and the pellet ressuspended in 1 mL of saline solution. Next, the suspension was sonicated (20 s with 22% of amplitude; Ultrasonic Processor, Cole-Parmer, Illinois, USA) to promote biofilm disruption. The number of colony forming units (CFU) in biofilm for each strain evaluated was determined by performing 10-fold serial dilutions in saline solution and plating in TSA in triplicate and incubating for 48 h.

Biofilm controls were cells not exposed to any antimicrobial agent tested. All experiments were carried out in triplicate and repeated three times.

Statistical Analysis

The data from all assays were compared using one-way analysis of variance (ANOVA) by applying Tukey's test with all calculations carried out using SPSS software (Statistical Package for the Social Sciences). Differences achieving a confidence level of 95 % were considered significant.

Results

Table 1 summarizes the values of vancomycin minimum inhibitory concentration obtained by E test and NAC minimum inhibitory concentration determined by the microbroth dilution method, for all the strains of staphylococci tested in this study. On the other hand, Table 2 shows the production of slime by Congo red agar and the expression of *icaD* gene in the strains of staphylococci evaluated. Slime production was detected in three strains (60 %) of *S. epidermidis* and in all *S. aureus* strains (100 %). The *icaD* gene was expressed in all strains of staphylococci evaluated.

Figures 1 and 2 present the number of viable cells (after exposure to the two antimicrobial agents tested alone) of *S. epidermidis* and *S. aureus* strains tested, respectively, expressed as log CFU/mL. The results evidenced that vancomycin alone causes no effect on the staphylococci strains evaluated, since the reduction promoted by this antibiotic was less than 3 $\log_{10}$. On the contrary, the use of NAC alone showed to reduce the number of cells in biofilms of staphylococci strains. In fact, the use of NAC 10 × MIC reduced significantly the cells in biofilm, evidencing a reduction of *circa* of 4-5 log CFU/mL ($p < 0.05$).

Figures 3 and 4 present the number of viable cells (after the treatment with the two agents in

Table 1. Values of minimal inhibitory concentration by E test (vancomycin) and microbroth dilution method (MBD) (NAC) for the staphylococci strains studied.

Strains	Number of laboratory identification	E test (vancomycin)	MBD (NAC)
S. epidermidis	9142	8 mg/mL	4 mg/mL
	1457	8 mg/mL	4 mg/mL
	1	3 mg/mL	8 mg/mL
	127	4 mg/mL	8 mg/mL
	165	3 mg/mL	8 mg/mL
S. aureus	2	2 mg/mL	8 mg/mL
	35	2 mg/mL	8 mg/mL
	73	1.5 mg/mL	8 mg/mL
	173	2 mg/mL	8 mg/mL
	174	2 mg/mL	8 mg/mL

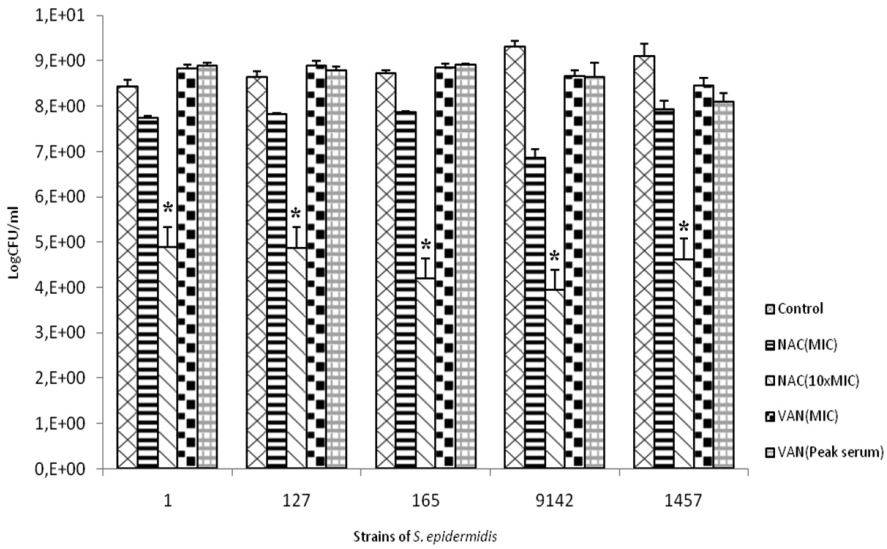

Figure 1. Effect of NAC and vancomycin (VAN) alone on biofilm cells of *S. epidermidis*. *Antimicrobial effect statistically different from untreated cells ($p < 0.05$).

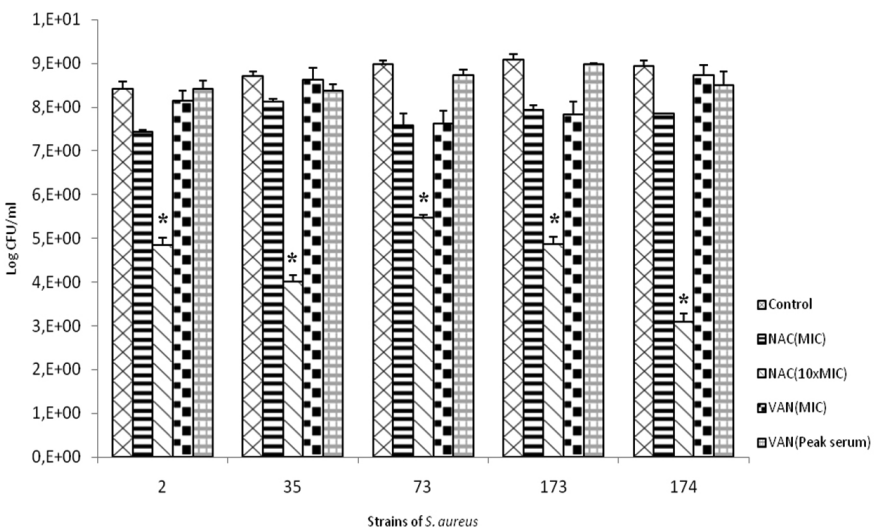

Figure 2. Effect of NAC and vancomycin (VAN) alone on biofilm cells of *S. aureus*. *Antimicrobial effect statistically different from untreated cells ($p < 0.05$).

Rev. Bras. Eng. Biom., v. 29, n. 2, p. 184-192, jun. 2013
Braz. J. Biom. Eng., 29(2), 184-192, June 2013

Table 2. Slime production determined by the method of Congo red agar and *icaD* gene expression.

Strains	Number of laboratory identification	Congo red agar (black or red colonies)	Gene expression of icaD (present or absent)
S. epidermidis	9142	Black	Present
	1457	Black	Present
	1	Red	Present
	127	Black	Present
	165	Red	Present
S. aureus	2	Black	Present
	35	Black	Present
	73	Black	Present
	173	Black	Present
	174	Black	Present

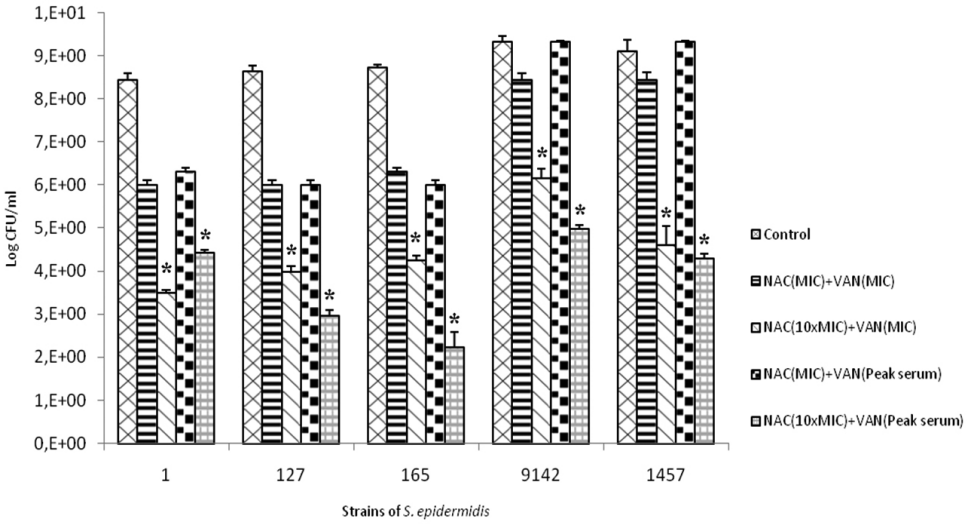

Figure 3. Effect of NAC and vancomycin (VAN) in combination on biofilm cells of *S. epidermidis*. *Combinations statistically different from control ($p < 0.05$).

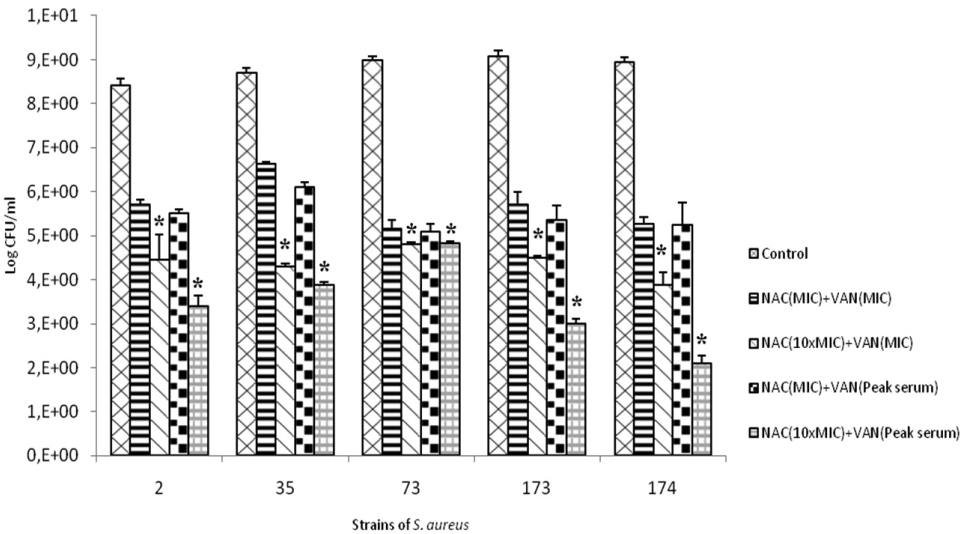

Figure 4. Effect of NAC and vancomycin (VAN) in combination on biofilm cells of *S. aureus*. *Combinations statistically different from control ($p < 0.05$).

combination) of *S. epidermidis* and *S. aureus* strains tested, respectively, expressed as log CFU/mL. It can be observed that the combination of NAC at 10 × MIC with vancomycin (independently of the concentration used) showed a notorious effect on biofilm cells of all strains of *S. epidermidis* and *S. aureus* evaluated, promoting CFU reductions of about 5-6 $\log_{10}$ ($p < 0.05$).

Discussion

Bacterial cells have grown in biofilm for billions of years, as a part of their successful strategy to colonize most of this planet and most of its life forms. In fact, this is the predominant mode of growth of bacteria in natural environment. Their distinct phenotype makes them resistant to antimicrobial agents, and their matrix makes them tolerant to the antibacterial molecules and immune system cells mobilized by the host. The antimicrobial molecules must diffuse through the biofilm matrix in order to inactivate the encased cells. Thus, the extracellular polymeric substances constituting this matrix present a diffusional barrier for these molecules by influencing either the rate of transport of the molecule to the interior of the biofilm or the reaction of the antimicrobial agent with the matrix material (Donlan and Costerton, 2002; Presterl *et al.*, 2009).

The ability of staphylococci cells to adhere and form biofilm is mediated by the production of the polysaccharide intercellular adhesin (PIA). Their synthesis is encoded by the gene product of ica locus of the operon *icaADBC*, being the product of this locus essential for biofilm formation and virulence of microorganisms (O'Toole *et al.*, 2000). Arciola *et al.* (2001) work corroborates with the former statement, showing in their studies that the gene *icaA* and *icaD* have an important role in biofilm formation of *S. epidermidis* and *S. aureus*. The *icaD* gene was evaluated in this study and was reported to be expressed in all strains of *S. epidermidis* (100%) and *S. aureus* (100 %) evaluated. Another indicator of virulence is the Congo red reaction that provides information about the ability of microorganisms to produce slime (Prpic *et al.*, 1983; Bernardi *et al.*, 2007). This study showed that three *S. epidermidis* strains (9142, 1457 and 127) and all *S. aureus* strains evaluated were slime producers.

Several authors have reported that the development of resistance is reduced when antibiotics are combined (Moellering, 2008; Perlroth *et al.*, 2008). Taking into consideration this notion and aiming to overcome the current problem of the emergence of resistance several researchers focused their studies in the use of combinations. This study evaluated the susceptibility of biofilm cells of *S. epidermidis* and *S. aureus* to NAC and vancomycin alone and in combination. NAC was choose due to its potential role in biofilm matrix being able to reduce the extracellular polysaccharide content of biofilm (Olofsson *et al.* 2003). Our results demonstrated that NAC-vancomycin exhibited a significant antimicrobial effect on biofilm cells of all strains studied. This was possibly due to the degradation of the matrix promoted by NAC which facilitated the penetration into the biofilm and the action of vancomycin being then able to reduce the viable number of bacterial cells (Dunne *et al.,* 1993; Wilcox *et al.*, 2001). As we have previously hypothesized this combination has a synergistic effect due to their different modes of action, acting into two different components of the biofilm namely the matrix and the cellular content. The use of vancomycin alone was ineffective against biofilm cells of the isolates studied, whereas NAC demonstrated a significant inhibitory action expressed by reductions of viability of *circa* of 4-5 $\log_{10}$ CFU/mL (Figures 1 and 2). As described by several authors, antibiotics at MIC concentration cause no killing effect on bacteria when growing in biofilm communities (Tetz *et al.*, 2009). Our results are in accordance with the previous researchers since NAC and vancomycin alone or in combination both at MIC concentration caused no killing effect on staphylococci cells, showing a viable cells reduction of less than 3 $\log_{10}$. On the contrary, when exposed to NAC-vancomycin (NAC 10 × MIC combined with vancomycin independently of the concentration used) a high antibactericidal activity was observed, reaching a reduction on viability cells of about 5-6 $\log_{10}$ CFU/mL. Other studies were performed with the objective to find new therapeutic strategies using combinatorial approaches and using NAC or vancomycin but no promising results were obtained. As example, Gomes and co-workers demonstrated that no synergy was observed combining farnesol with vancomycin (Gomes *et al.*, 2011) and NAC with farnesol (Gomes *et al.*, 2012) on biofilms of *S. epidermidis*. The combination of NAC and tigecycline on *S. epidermidis* biofilms was reported by Aslam *et al.* (2007) and using NAC at 20 × MIC (80 mg/mL) and tigecycline at 1000 × MIC (1 mg/mL), neither synergy nor an effective activity was also observed (reduction of less than 3 $\log_{10)}$. On the other hand and supporting our results Abbas *et al.* (2012) studied the effect of NAC on biofilm cells of *P. aeruginosa* and showed that NAC has a moderate effect against this pathogen.

Zhao and Liu (2010) also proved the inhibitory effect of this compound against the previous microorganism expressed by the antibacterial activity and detachment of biofilm cells and defend that its action is caused by competitively inhibiting cysteine utilization in bacteria or by reaction of its sulfhydryl group with bacterial cell proteins. El-Feky *et al.* (2009) investigated the effect of NAC (2 and 4mg/mL) in combination with ciprofloxacin (MIC and 2 × MIC) against biofilm cells of several bacteria including *S. aureus*, *S. epidermidis*, *E. coli*. and others. The authors concluded that NAC potentiate the therapeutic action of ciprofloxacin. This was due to the ability of NAC to degrade the extracellular polysaccharide matrix of biofilms. As general conclusion they suggest the potential use of ciprofloxacin-NAC combination as therapeutic strategy against bacterial infections mediated by biofilms.

In general, it is believed that NAC is able of acting at the level of the matrix promoting the increasing therapeutic efficacy of vancomycin, which alone had no significant effect on cells in biofilms. This activity of NAC can result in the detachment of cells, individually or in clusters, becoming the biofilm and detached cells more susceptible to the action of other antimicrobial agents and to the immune system. Thus, the role of NAC and other antimicrobial agents targeting matrix and promoting this mechanism of detachment of cells can be an important help in the eradication of biofilms associated infections.

In conclusion, the results of the present study show excellent activity *in vitro* of NAC (10 × MIC) alone and in combination with vancomycin (MIC or peak serum) on *S. epidermidis* and *S. aureus* biofilms. The results indicate that this combination may represents a novel therapeutic option for infections caused by staphylococci, especially by strains able to form biofilm on biotic or abiotic surfaces, because the use of antimicrobial agents in combination as described by other authors reduces the development of resistance.

Acknowledgements

The authors acknowledge the financial support from CAPES, Microbiology Laboratory/NAC (UNESP/FCF/CAr, Araraquara, Brazil) and Molecular Biology Laboratory (UNESP/Faculty of Preventive Veterinary Medicine, Jaboticabal, Brazil).

References

Abbas HA, Serry FM, El-Masry EM. Combating *Pseudomonas aeruginosa* biofilms by potential biofilm inhibitors. Asian Journal of Research in Pharmaceutical Sciences. 2012; 2:66-72.

Arciola CR, Baldassarri L, Montanaro L. Presence of *icaA* and *icaD* genes and slime production in a collection of *Staphylococcal* strains from catheter-associated infections. Journal of Clinical Microbiology. 2001; 39:2151-6. PMid:11376050 PMCid:88104. http://dx.doi.org/10.1128/JCM.39.6.2151-2156.2001

Aslam S, Trautner BW, Ramanathan V, Darouiche RO. Combination of tigecycline and N-acetylcysteine reduces biofilm-embedded bacteria on vascular catheters. Antimicrobial Agents Chemotherapy. 2007; 51:1556-8. PMid:17220399 PMCid:1855497. http://dx.doi.org/10.1128/AAC.00893-06

Bernardi ACA, Pizzolitto EL, Pizzolitto AC. Detecção da produção de slime por estafilococos coagulase-negativa isolados de cateter venoso central. Revista Ciências Farmacêuticas Básica e Aplicada. 2007; 28(1):57-66.

Blaser J, Vergeres P, Widmer AF, Zimmerli W. *In vivo* verification of *in vitro* model of antibiotic treatment of device-related infection. Antimicrobial Agents Chemotherapy. 1995; 39:1134-9. http://dx.doi.org/10.1128/AAC.39.5.1134

Boles BR, Horswill AR. Staphylococcal biofilm disassembly. Trends in Microbiology. 2011; 19(9):449-55. PMid:21784640 PMCid:3164736. http://dx.doi.org/10.1016/j.tim.2011.06.004

Cargill JS, Upton M. Low concentration of vancomycin stimulate biofilm formation in some clinical isolates of *Staphylococcus epidermidis*. Journal of Clinical Pathology. 2009; 62:1112-6. PMid:19640858. http://dx.doi.org/10.1136/jcp.2009.069021

Cerca N, Martins S, Cerca F, Jefferson KK, Pier GB, Oliveira R, Azeredo J. Comparative assessment of antibiotic susceptibility of coagulase-negative staphylococci in biofilm versus planktonic culture as assessed by bacterial enumeration or rapid XTT colorimetry. Journal of Antimicrobial Chemotherapy. 2005; 56:331-6. PMid:15980094 PMCid:1317301. http://dx.doi.org/10.1093/jac/dki217

Chambers ST. Diagnosis and management of staphylococcal infections of pacemakers and cardiac defibrillators. Internal Medicine Journal. 2005; 35:63-71. PMid:16271062. http://dx.doi.org/10.1111/j.1444-0903.2005.00980.x

Charles PGP, Ward PB, Johnson PDR, Howden BP, Grayson ML. Clinical features associated with bacteremia due to heterogeneous vancomycin-intermediate *Staphylococcus aureus*. Clinical Infectious Diseases. 2004; 38:448-51. PMid:14727222. http://dx.doi.org/10.1086/381093

Chuard C, Herrmann M, Vaudaux P, Wadvogel FA. Successful therapy of experimental chronic foreign-body infection due to methicillin-resistant *Staphylococcus aureus* by antimicrobial combinations. Antimicrobial Agents Chemotherapy. 1991; 35:2611-6. http://dx.doi.org/10.1128/AAC.35.12.2611

Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: A common cause of persistent infections. Science. 1999; 284:1318-22. PMid:10334980. http://dx.doi.org/10.1126/science.284.5418.1318

Costerton W, Montanaro L, Balaban N, Arciola CR. Prospecting gene therapy of implant infections. International Journal Artificial Organs. 2009; 32:689-95. PMid:19882546.

Cramton S, Gotz F. Biofilm development in *Staphylococcus*. Microbial Biofilms. 2004; 64-84.

Davis JS. Management of bone and joint infections due to *Staphylococcus aureus*. Internal Medicine Journal. 2005; 35: 79-96. PMid:16271064. http://dx.doi.org/10.1111/j.1444-0903.2005.00982.x

Domínguez-Herrera J, Docobo-Pérez F, López-Rojas R, Pichardo C, Ruiz-Valderas R, Lepe JA, Pachón J. Efficacy of daptomycin versus vancomycin in an experimental foreign-body and systemic infection model caused by biofilm-producers and methicillin-resistant *Staphylococcus epidermidis*. Antimicrobial Agents and Chemotherapy. 2012. 56:613-7. PMid:22123684 PMCid:3264234. http://dx.doi.org/10.1128/AAC.05606-11

Donlan RM, Costerton JW. Biofilms: Survival mechanisms of clinically relevant microorganisms. Clinical Microbiology Reviews. 2002; 15:167-93. http://dx.doi.org/10.1128/CMR.15.2.167-193.2002

Dunne WMJ, Mason EOJ, Kaplan SL. Diffusion of rifampin and vancomycin through a *Staphylococcus epidermidis* biofilm. Antimicrobial Agents Chemotherapy. 1993; 37:2522-6. http://dx.doi.org/10.1128/AAC.37.12.2522

El-Feky MA, El-Rehewy MS, Hassan MA, Abolella HA, Abd El-Baky RM, Gad GF. Effect of ciprofloxacin and *N*-acetylcysteine on bacterial adherence and biofilm formation on ureteral stent surfaces. Polish Journal of Microbiology 2009; 58:261-7. PMid:19899620.

Gagnon RF, Richards GK, Subang R. Experimental *Staphylococcus epidermidis* implant infection in the mouse: Kinetics of rifampin and vancomycin action. Asaio Journal. 1992; 38:M596-9. PMid:1457930. http://dx.doi.org/10.1097/00002480-199207000-00106

Gomes F, Leite B, Teixeira P, Azeredo J, Oliveira R. Farnesol in combination with *N*-acetylcysteine against *Staphylococcus epidermidis* planktonic and bioflm cells. Brazilian Journal of Microbiology. 2012:235-42. http://dx.doi.org/10.1590/S1517-83822012000100026

Gomes F, Leite B, Teixeira P, Cerca N, Azeredo J, Oliveira R. Farnesol as antibiotics adjuvant in *Staphylococcus epidermidis* control *in vitro*. American Journal of the Medical Sciences. 2011; 341:191-5. PMid:21107231. http://dx.doi.org/10.1097/MAJ.0b013e3181fcf138

Gosbell IB. Diagnosis and management of catheter-related blood-stream infections due to *Staphylococcus aureus*. Internal Medicine Journal. 2005; 35:45-62. PMid:16271061. http://dx.doi.org/10.1111/j.1444-0903.2005.00979.x

Howden BP, Ward PB, Charles PG, Korman TM, Fuller A, Du Cros P, Grabsch EA, Roberts J, Read K, Bak N, Hurley J, Johnson PD, Morris AJ, Mayall BC, Grayson ML. Treatment outcomes for serious infections caused by methicillin-resistant *Staphylococcus aureus* with reduced vancomycin susceptibility. Clinical Infect Diseases. 2004; 38:521-8. PMid:14765345. http://dx.doi.org/10.1086/381202

Kloos WE, Bannerman TL. Update on clinical significance of coagulase-negative staphylococci. Clinical Microbiology. 1994; 7:117-40.

Lowy FD. *Staphylococcus aureus* infections. New England Journal of Medicine. 1998; 339:520-32. PMid:9709046. http://dx.doi.org/10.1056/NEJM199808203390806

Lucet JC, Herrmann M, Rohner P, Auckenthaler R, Waldvogel FA, Lew DP. Treatment of experimental foreign body infection caused by methicillin-resistant *Staphylococcus aureus*. Antimicrobial Agents Chemotherapy. 1990; 34:2312-7. http://dx.doi.org/10.1128/AAC.34.12.2312

Mah TF, O'Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. Trends in Microbiology. 2001; 9:34-9. http://dx.doi.org/10.1016/S0966-842X(00)01913-2

McCann MT, Gilmore BF, Gorman SP. *Staphylococcus epidermidis* device-related infections: pathogenesis and clinical management. Journal of Pharmacy and Pharmacology. 2008; 60:1551-71. PMid:19000360.

Moellering RC. Current treatment options for community-acquired methicillin-resistant *Staphylococcus aureus* infection. Clinical Infectious Diseases, 2008; 46:1032-7. PMid:18444820. http://dx.doi.org/10.1086/529445

Olofsson AC, Hermansson M, Elwing H. N-acetyl-l-cysteine affects growth, extracellular polysaccharide production, and bacterial biofilm formation on solid surfaces. Applied and Environmental Microbiology. 2003; 69:4814-22. PMid:12902275 PMCid:169071. http://dx.doi.org/10.1128/AEM.69.8.4814-4822.2003

O'Toole G, Kaplan HB, Kolter R. Biofilm formation as microbial development. Annual Review Microbiology. 2000; 54:49-79. PMid:11018124. http://dx.doi.org/10.1146/annurev.micro.54.1.49

Otto M. Staphylococcal biofilms. Current Topics in Microbiology and Immunology. 2008; 322:207-28. PMid:18453278 PMCid:2777538. http://dx.doi.org/10.1007/978-3-540-75418-3_10

Perez-Giraldo C, Rodriguez-Benito A, Moran FJ, Hurtado C, Blanco MT, Gómez-García AC. Influence of N-acetylcysteine on the formation of biofilm by *Staphylococcus epidermidis*. Journal of Antimicrobial Chemotherapy. 1997; 39:643-6. PMid:9184365. http://dx.doi.org/10.1093/jac/39.5.643

Perlroth J, Kuo M, Tan J, Bayer AS, Miller LG. Adjunctive use of rifampin for the treatment of *S. aureus* infections. Archives of Internal Medicine. 2008; 168:805-19. PMid:18443255. http://dx.doi.org/10.1001/archinte.168.8.805

Pootoolal J, Neu J, Wright GD. Glycopeptide antibiotic resistance. Annual Review Pharmacology and Toxicology. 2002; 42:381-408. PMid:11807177. http://dx.doi.org/10.1146/annurev.pharmtox.42.091601.142813

Presterl E, Hadju S, Lassnigg AM, Hirschl AM, Holinka J, Graninger W. Effects of azithromycin in combination with vancomycin, daptomycin, fosfomycin, tigecycline, and ceftriaxone on Staphylococcus epidermidis biofilms. Antimicrobial Agents and Chemotherapy. 2009; 53:3205-10. PMid:19451280 PMCid:2715620. http://dx.doi.org/10.1128/AAC.01628-08

Prpic JK, Robins-Browne RM, Davey RB. Differentiation between virulent and avirulent *Yersinia enterocolitica* isolates by using Congo red agar. Journal of Clinical Microbiology. 1983; 18:486-90. PMid:6630438 PMCid:270839.

Tetz GV, Artemenko NK, Tetz VV. Effect of DNase and antibiotics on biofilm characteristics. Antimicrobial Agents Chemotherapy. 2009; 53:1204-9. PMid:19064900 PMCid:2650517. http://dx.doi.org/10.1128/AAC.00471-08

Wilcox MH, Kite P, Mills K, Sugden S. In situ measurement of linezolid and vancomycin concentrations in intravascular catheter-associated biofilm. Journal of Antimicrobial Chemotherapy. 2001; 47:171-5. PMid:11157901. http://dx.doi.org/10.1093/jac/47.2.171

Zhao T, Liu Y. N-acetyl cysteine inhibit biofilms produced by *Pseudomonas aeruginosa*. BMC Microbiology. 2010; 10:140. PMid:20462423 PMCid:2882372. http://dx.doi.org/10.1186/1471-2180-10-140

Authors

Bruna Leite, Clovis Souza, Elisabeth Pizzolitto
Department of Biotechnology, Federal University of São Carlos – UFSCar, Rodovia Washington Luis, Km 235, CEP 13560-000, São Carlos, SP, Brazil.

Fernanda Gomes, Pilar Teixeira, Rosário Oliveira
Institute for Biotechnology and Bioengineering – IBB, University of Minho, Braga, Portugal.

Poliana Melo
Faculty of Preventive Veterinary Medicine, São Paulo State University – USP, Jaboticabal, SP, Brazil.

Elisabeth Pizzolitto*
Faculty of Pharmaceutical Sciences, São Paulo State University – UNESP, Rua Expedicionários do Brasil, 1621, CEP 14801-360, Araraquara, SP, Brazil.