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ORIGINAL ARTICLE

# Antimicrobial synergism against different lineages of methicillin-resistant *Staphylococcus aureus* carrying SCC*mec* IV

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biofilm, biomass, drug synergism, MRSA, SCC*mec* IV.

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## Abstract

**Aim:** To evaluate the synergistic activity of antimicrobial drugs against lineages of methicillin-resistant *Staphylococcus aureus* (MRSA) carrying SCC*mec* IV. The biofilm production and related genes were also detected.

**Methods and Results:** Forty two MRSA isolates were tested for biofilm production and related genes. Biofilm/biomass susceptibility to gentamicin (G), linezolid (L), rifampicin (R) and vancomycin (V) was determined for six isolates from three lineages prevalent in Rio de Janeiro hospitals in concentrations ranging from 0·25 to 64 $\mu$g ml$^{-1}$. Biomass was evaluated by microtitre plate test and number of viable cells (CFU cm$^{-2}$) and inspected by epifluorescence microscopy. All isolates presented the *ica*A and *sas*G genes, but only 38% were biofilm producers. There were 50 and 45% biomass reductions when concentrations ≥4 $\mu$g ml$^{-1}$ of R or L and ≥16 $\mu$g ml$^{-1}$ of G or V, respectively, were used. Synergism tests produced a 55% biomass reduction with $R_{2\mu g\,ml^{-1}} + G_{16\mu g\,ml^{-1}}$, $R_{2\mu g\,ml^{-1}} + L_{2\mu g\,ml^{-1}}$, $R_{2\mu g\,ml^{-1}} + V_{4\mu g\,ml^{-1}}$, and $L_{2\mu g\,ml^{-1}} + V_{4\mu g\,ml^{-1}}$. Number of viable cells was reduced from 2 to 3 logs with $R_{2\mu g\,ml^{-1}} + L_{2\mu g\,ml^{-1}}$ and $R_{2\mu g\,ml^{-1}} + V_{4\mu g\,ml^{-1}}$.

**Conclusions:** Synergisms involving R *plus* L and R *plus* V caused important reductions in biofilm/biomass and the number of viable cells. Drug combinations should be considered in the chemotherapies of MRSA-SCC*mec* IV infections.

**Significance and Impact of the Study:** Biofilms in MRSA infections restrict the clinical choice of antimicrobials. Thus, knowledge of the best options for monotherapy and drug synergisms could improve clinical results.

## Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen (David and Daum 2010). MRSA isolates present a penicillin-binding protein, PBP2a, encoded by the *mec*A gene that is inserted in a mobile genetic element called the staphylococcal cassette chromosome *mec* (SCC*mec*) (Katayama *et al.* 2000). Among the twelve different SCC*mec* allotypes already described (IWG-SCC 2013), type IV, which is related to the community isolates, has been emerging in hospitals worldwide causing infections in patients with or without traditional risk factors for MRSA (Schuenck *et al.* 2009; Holzknecht *et al.* 2010; Lesosky *et al.* 2011; Caboclo *et al.* 2013; Velasco *et al.* 2012).

In Brazil, MRSA isolates related to the Brazilian endemic clone (BEC)/sequence type (ST) 239 are traditionally found in hospital-acquired infections (Santos *et al.* 1999). However, over the last decade, changes in hospital epidemiology have been observed, with the emergence of

nosocomial infections caused by SCC*mec* IV isolates, including such predominant lineages as USA400/ST1 (MW2 clone), USA1100/ST30 (Ocean Pacific clone) and USA800/ST5 (paediatric clone) (Silva-Carvalho *et al.* 2009; Schuenck *et al.* 2012; Caboclo *et al.* 2013). Our group showed the polyclonal emergency of nonmultiresistant MRSA-SCC*mec* IV isolates in health-care-associated infections causing deaths in patients in a hospital in Rio de Janeiro (Schuenck *et al.* 2009).

Biofilm formation by *Staph. aureus* isolates is common in medical devices such as catheters and prostheses, allowing the pathogen to resist host immune responses and antimicrobials (Donlan and Costerton 2002). However, some studies have shown that the majority of type IV isolates do not produce biofilms or are weak or moderate biofilm formers (Cha *et al.* 2011; Schuenck *et al.* 2012). Moreover, the action of antimicrobial agents either individually or in combination against MRSA-SCC*mec* IV isolates has not yet been analysed. Thus, this study aimed to evaluate the activity of antimicrobial agents alone and in various combinations against the biofilm/biomass produced by different lineages of MRSA-SCC*mec* IV isolates from hospitals in Rio de Janeiro. Biofilm production and related genes were also evaluated.

## Materials and methods

### Clinical isolates

Of a collection of 128 MRSA-SCC*mec* IV isolates previously characterized as to species (Bannerman and Peacock 2007), methicillin resistance (CLSI 2012) and SCC*mec* type (Milheiriço *et al.* 2007), 42 were selected for this study. This selection was based on the DNA polymorphism profiles determined by the pulsed-field gel electrophoresis (PFGE) (Vivoni *et al.* 2006) and multilocus sequence typing (MLST) methods (Enright *et al.* 2000). The lineages were classified according to McDougal *et al.* (2003) as USA800/ST5 (14 isolates), USA400/ST1 (9) and USA1100/ST30 (4); the remaining 15 isolates belonged to other STs and/or clonalities. These isolates were obtained between July 2004 and November 2008 from different clinical specimens from patients in eight hospitals in Rio de Janeiro city (Table 1).

### Biofilm/biomass formation assay

Biofilm/biomass formation was evaluated for all 42 MRSA-SCC*mec* IV isolates according to the microtitre plate test protocol modified from that described by Stepanovic *et al.* (2000). A bacterial suspension in Tryptic Soy Broth (TSB, Merck, Algés, Portugal) supplemented with 1% glucose and adjusted to a final concentration of *c.* $1 \times 10^7$ cells $ml^{-1}$ was transferred to a microtitre plate (200 $\mu$l per well). The plates were incubated aerobically on a horizontal shaker at 120 rpm at 37°C. After 24 h, the content of each well was removed and the wells were washed twice with 200 $\mu$l of sterile water. The plates were air-dried for 20 min, and bacterial biomass adhering to the inner surfaces of each microtitre plate well was fixed with 200 $\mu$l of 98% metanol (Vaz Pereira, Portugal) per well during 15 min. Afterwards, the plates were emptied,

**Table 1** General characteristics and biofilm formation ability of 42 MRSA-SCC*mec* IV isolates, positive for *ica*A and *sas*G genes, isolated from hospitals in Rio de Janeiro

| Sequence type by MLST (*n*) | Clonality by PFGE | Clinical source (*n*) | Biofilm production (*n*) |
|---|---|---|---|
| 1 (9) | USA400 | Prosthesis secretion (3), urine (3), nasal (1), ear secretion (1), bronchial alveolar lavage (1) | + (1), − (8) |
| 5 (14) | USA800 | Nasal (4), wound (4), prosthesis secretion (3), bronchial alveolar lavage (2), blood (1) | ++ (2), + (6), − (6) |
| 30 (4) | USA1100 | Wound (2), bone secretion (1), renal abscess (1) | + (2), − (2) |
| 5 (2) | ND | Nasal (2), | ++ (1), + (1) |
| 1203 (2) | ND | Catheter tip (1), wound (1) | − (2) |
| 97 (3) | ND | Pleural fluid (1), nasal (2) | ++ (1), − (2) |
| 8 (1) | USA 300 | Wound | ++ |
| 22 (1) | EMRSA-15 | Tracheal secretion | − |
| 45 (1) | USA 600 | Nasal | − |
| 72 (1) | ND | Blood | − |
| 2102 (1) | ND | Tracheal secretion | − |
| 714 (1) | ND | Wound | + |
| 30 (1) | ND | Bronchial alveolar lavage | − |
| 2112 (1) | ND | Nasal | − |

MLST, multilocus sequence typing; PFGE, pulsed-field gel electrophoresis; ND, not determined; strong biofilm producer (+++), moderate biofilm producer (++), weak biofilm producer (+) and nonbiofilm producer (−).

left to dry for 20 min, and fixed biomass was stained for 5 min with 200 μl of crystal violet (CV) (Merck) per well. Excess stain was rinsed off by placing the plate under running tap water. After the plate was air-dried, the dye bound to the adherent cells was resuspended with 200 μl of 33% (v/v) glacial acetic acid (Merck) per well. The optical density (OD) of the obtained solution was measured at 570 nm using a microtitre plate reader (Tecan, Model Sunrise-basic Tecan, Grödig, Austria), and the ability of *Staph. aureus* isolates to produce biofilm/biomass was evaluated as $OD_{570}$ values.

The *Staphylococcus epidermidis* strains, ATCC 35984 (strong biofilm producer) and ATCC12228 (nonbiofilm producer) were used as controls. All isolates were classified in terms of their ability to form biofilm based on absorbance values into the following categories: strong producer (+++, OD > 2·0), moderate producer (++, OD from 1·0 to 2·0), weak producer (+, OD from 0·5 to <1·0) and non producer (−, OD < 0·5) of biofilm.

### PCR method to detect biofilm-related genes

PCR was performed for the 42 MRSA-SCC*mec* IV isolates to detect *ica*A (Mirani and Jamil 2011) and *sas*G (Roche *et al.* 2003) genes, which encode a cell surface protein related to biofilm and the polysaccharide intercellular adhesin (PIA), respectively. The primer sequences used were ICAAF – 5′- AAACTTGGTGCGGTTACAGG-3′ and ICAAR – 5′-TCTGGGCTTGACCATGTTG-3′ (*ica*A gene), and SASGF – 5′-GAGATAAGAAAGGACCGG and SASGR – TTAATTCTTTCTTCTACG-3′ (*sas*G gene).

### Bacterial biomass susceptibility testing

Biomass susceptibility to gentamicin (G), linezolid (L), rifampicin (R) and vancomycin (V) (Sigma-Aldrich, St. Louis, MO) were determined for six isolates selected from three prevalent lineages: USA400/ST1 (isolates 633, weak biofilm producer, and 915, nonproducer), USA800/ST5 (isolates 1112, weak biofilm producer, and 1177, moderate biofilm producer) and USA1100/ST30 (isolates 943, weak biofilm producer, and 1314, nonproducer). After biomass formation for 24 h, the isolates were exposed to antimicrobials, either individually or in combination, with concentrations ranging from 0·25 to 64 $\mu g\ ml^{-1}$. The concentrations used were based on the antimicrobial break points (CLSI 2012). After antibiotic treatment for 24 h, the biofilms were characterized in terms of their biomass through the microtitre plate test (above described) and the number of cultivable cells, by plate count agar (CFU $cm^{-2}$). To determine the number of CFU, the biomass formed within the wells was removed by rapid sonication for 6 min and, subsequently, serially diluted. After plating the serial dilution on Tryptic Soy Agar (TSA, Merck), the plates were incubated at 37°C, in an aerobic incubator for 18 h prior to enumeration.

### Statistical methods

Data were recorded as the mean standard deviation. Because of the small sample size and the abnormal distribution, the Kruskal–Wallis test was used for multiple comparison analysis. Statistical significance was set at $P < 0{\cdot}05$. Data were analysed using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL).

## Results

### Biofilm formation assay and related genes

The biofilm production was considered positive only for 38% of MRSA-SCC*mec* IV isolates, being 8 USA800/ST5 isolates (six weak biofilm producers and two moderate), 1 USA400/ST1 isolate that was considered a weak biofilm producer, 2 USA1100/ST30 isolates that were detected as weak biofilm producers and five other isolates from other clonalities were two weak biofilm producers and three moderate (Table 1). The 42 MRSA isolates presented the expected amplification bands of 751 bp and 300 bp for *icaA* and *sasG* genes, respectively (data not shown).

### Biomass susceptibility testing

To assess the activity of antimicrobials against biofilm/biomass, drugs to which the MRSA-SCC*mec* IV isolates presented susceptibility in disc diffusion tests (data not shown) and that are usually indicated for treatment of MRSA infections were selected (Colli *et al.* 2007). After exposure to concentrations equal to or >4 $\mu g\ ml^{-1}$ of R or L, a biomass reduction of 50% was observed. For G or V, a reduction of about 45% was verified using concentrations ≥16 $\mu g\ ml^{-1}$ for all six MRSA-SCC*mec* IV isolates evaluated, irrespective of the lineage (Fig. 1). Regarding the cell viability analysis, which was determined by log CFU $cm^{-2}$, a reduction of up to 1 log for $V_{64\mu g\,ml^{-1}}$ or $R_{64\mu g\,ml^{-1}}$ or 2 log for $G_{64\mu g\,ml^{-1}}$ or $L_{64\mu g\,ml^{-1}}$ was observed for all isolates (Fig. 2).

The synergism experiments showed a biomass reduction of 55% for the antimicrobial associations $Rif_{2\mu g\,ml^{-1}}$ + $Gen_{16\mu g\,ml^{-1}}$, $Rif_{2\mu g\,ml^{-1}}$ + $Lin_{2\mu g\,ml^{-1}}$, $Rif_{2\mu g\,ml^{-1}}$ + $Van_{4\mu g\,ml^{-1}}$, and $Lin_{2\mu g\,ml^{-1}}$ + $Van_{4\mu g\,ml^{-1}}$ (Fig. 3), with results equivalent to those obtained with the drugs used alone but in higher concentrations (Fig. 4). In terms of cell viability, the synergistic combinations were also more effective when compared to the individual antimicrobial tests, with reductions of 2–3 logs when the antimicrobial

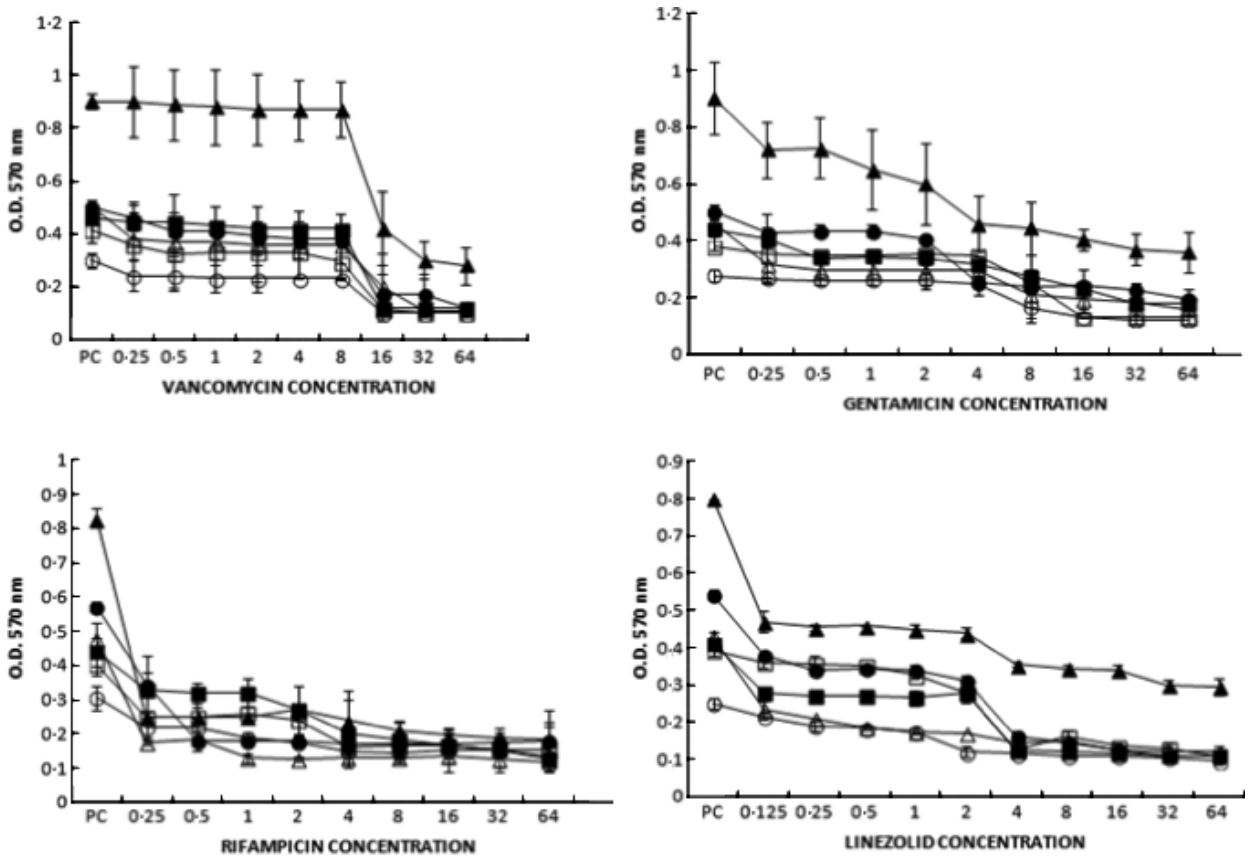


**Figure 1** Biomass evaluated after the use of increasing concentrations of antimicrobial agents (0·25–64 $\mu$g ml) for the six isolates from three MRSA-SCC*mec* IV lineages in relation to the positive control (PC) without antimicrobial exposure. Filled squares, triangles and circles represent 633 (USA400/ST1), 1177 (USA800/ST5) and 943 (USA1100/ST30) biofilm producer isolates; open squares, circles and triangles represent 915 (US400/ST1) and 1314 (USA1100/ST30) nonbiofilm producer isolates, and 1112 (USA800/ST5) weak producer isolate. (–■–) 633 (USA400/ST1); (–□–) 915 (US400/ST1); (–▲–) 1177 (USA800/ST5); (–△–) 1112 (USA800/ST5); (–●–) 943 (USA1100/ST30) and (–○–)1314 (USA1100/ST30).

combinations $Lin_{2\mu g\,ml^{-1}}$ + $Rif_{2\mu g\,ml^{-1}}$ and $Rif_{2\mu g\,ml^{-1}}$ + $Van_{4\mu g\,ml^{-1}}$ were used (Fig. 5). Although an increased activity of some drug associations has been shown compared with drugs alone against MRSA-SCC*mec* IV isolates, no differences between lineages and between drugs used were found ($P > 0{\cdot}05$).

## Discussion

Only a few studies have evaluated the action of different antimicrobials against MRSA isolates, including those carrying SCC*mec* IV (Silva-Carvalho *et al.* 2009; Schuenck *et al.* 2012). However, there are no studies that have evaluated the combined action of antimicrobial agents against certain MRSA-SCC*mec* IV lineages, which are nowadays emerging in hospitals worldwide. In this study, for the first time, the antimicrobial susceptibility of biofilm/biomass produced by type IV lineages of MRSA that are becoming prevalent in hospitals in Rio de Janeiro was evaluated.

Initially, our survey on biofilm formation found that the different lineages of MRSA-SCC*mec* IV evaluated were not strong biofilm producers. These results correspond to studies of other authors that have shown that the majority of type IV isolates are nonbiofilm producers (Cha *et al.* 2011; Schuenck *et al.* 2012). Cha *et al.* (2011) analysed 50 MRSA-type IV isolates and classified 86% as nonbiofilm and weak biofilm producers. In a previous study by our group, Schuenck *et al.* (2012) analysed 28 MRSA isolates from an orthopaedic hospital and classified all the type IV isolates (STs 1, 5 and 30) as weak or moderate biofilm producers, whereas only the isolates from the Brazilian clone/type III were considered to be strong biofilm producers. In fact, according to Kaito *et al.* (2011), MRSA-SCC*mec* IV isolates do not present a *psm-mec* region in the *mec* cassette, which is related to a higher capacity of biofilm formation. Additionally, in the present study, the ST5 isolates showed a greater tendency to be biofilm producers (eight isolates/$n$ = 14) than the

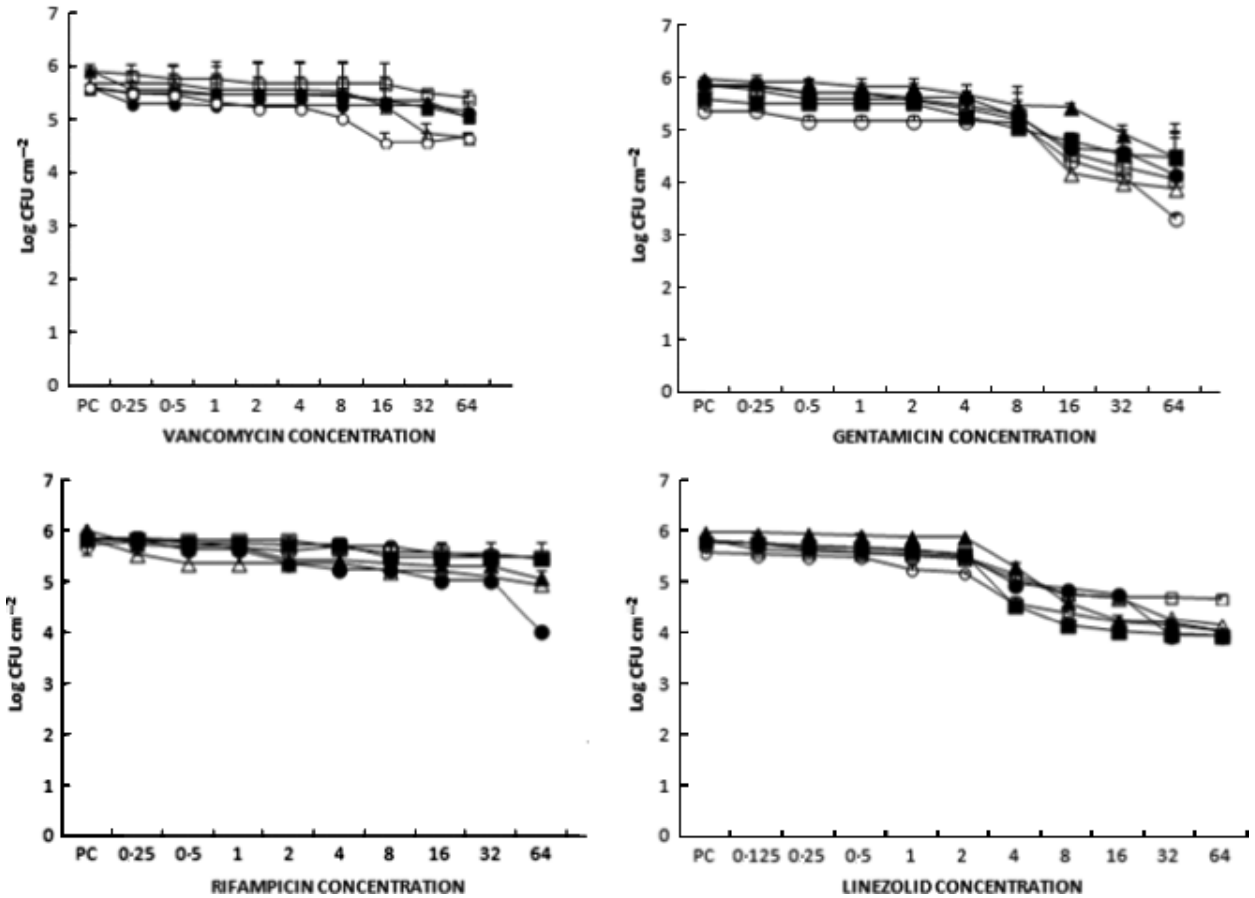


**Figure 2** Cell viability (log CFU $cm^{-2}$) evaluated after the use of increasing concentrations of antimicrobial agents (0·25–64 $\mu g\ ml^{-1}$) for the 6 isolates from three MRSA-SCC*mec* IV lineages in relation to the positive control (PC) without antimicrobial exposure. Filled squares, triangles and circles represent 633 (USA400/ST1), 1177 (USA800/ST5) and 943 (USA1100/ST30) biofilm producer isolates; open squares, circles and triangles represent 915 (US400/ST1) and 1314 (USA1100/ST30) nonbiofilm producer isolates, and 1112 (USA800/ST5) weak producer isolate. (–■–) 633 (USA400/ST1); (–□–) 915 (US400/ST1); (–▲–) 1177 (USA800/ST5); (–△–) 1112 (USA800/ST5); (–●–) 943 (USA1100/ST30) and (–○–)1314 (USA1100/ST30).

isolates of ST1 lineage (1/9). This fact may be related to the origin of this lineage (paediatric clone), indicating that these isolates are more adapted to clinical environments. However, further studies involving more MRSA isolates from this and other lineages and other regions are necessary.

It is known that *Staph. aureus* has a great capacity to bind to different surfaces, including plastics, maintaining colonization through various forms of adhesins (Otto 2012). Thus, it was possible to evaluate the biomass formed and the number of bacterial cells present there. Furthermore, the action of the antimicrobials against the biofilms/biomass was similar to all isolates, irrespective of their ability to produce or not biofilm. Linezolid and rifampicin antibiotics were observed to be better for reducing biomass than gentamicin or vancomycin ones. Saginur *et al.* (2006) evaluated the biofilm antimicrobial susceptibility of 12 MRSA isolates and found that rifampicin was the single most active agent. Fernández-Barat *et al.* (2012) evaluated the effects of systemic treatment with linezolid compared with vancomycin on biofilm formation, in mechanically ventilated pigs with severe MRSA-induced pneumonia. They observed that the lowest bacterial burden was found in endotracheal tubes treated with linezolid in comparison with the untreated endotracheal tubes or with those treated with vancomycin, confirming the findings of the present study.

The observations in the present study concerning the limited activity of vancomycin against staphylococcal cells embedded in biofilm/biomass are consistent with other studies. (Raad *et al.* 2007; Wells *et al.* 2011). Wells *et al.* (2011) evaluated the viability of staphylococcal cells in mechanically dispersed biofilms and biomass formation after treatment with vancomycin; in two biofilm producer isolates cultivated in silk suture, a significant reduction in cells viability was observed only at high concentrations of

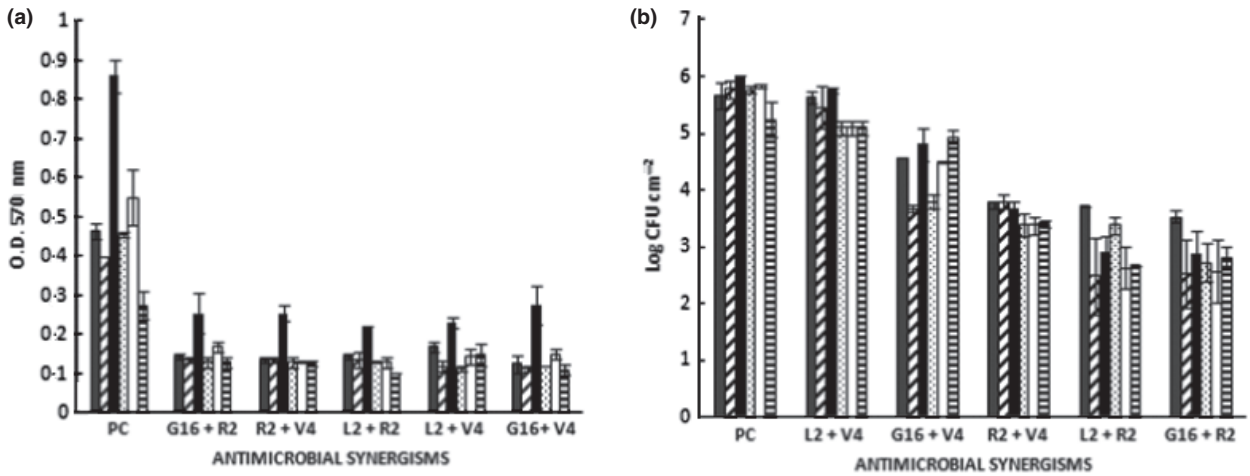


**Figure 3** Biomass (a) and cell viability (log CFU $cm^{-2}$) (b) evaluated after the synergistic activity of antimicrobials at different concentrations ($\mu g\ ml^{-1}$) for the six isolates from three MRSA-SCC*mec* IV lineages in relation to the positive control (PC) without antimicrobial exposure. $G_{16}$–16 $\mu g\ ml^{-1}$ of gentamicin; $V_4$–4 $\mu g\ ml^{-1}$ of vancomycin, $L_2$–2 $\mu g\ ml^{-1}$ of linezolid and $R_2$–2 $\mu g\ ml^{-1}$ of rifampicin. Grey bars represent the 633 (USA400/ST1) isolate, diagonal striped bars represent the 915 (USA400/ST1) isolate, black bars represent the 1177 (USA800/ST5) isolate, dotted bars represent the 1112 (USA800/ST5) isolate, white bars represent the 943 (USA1100/ST30) isolate, and horizontal striped bars represent the 1314 (USA1100/ST30) isolate. (■) 633 (USA400/ST1); (▨) 915 (US400/ST1); (■) 1177 (USA800/ST5); (▦) 1112 (USA800/ST5); (□) 943 (USA1100/ST30) and (▤)1314 (USA1100/ST30).

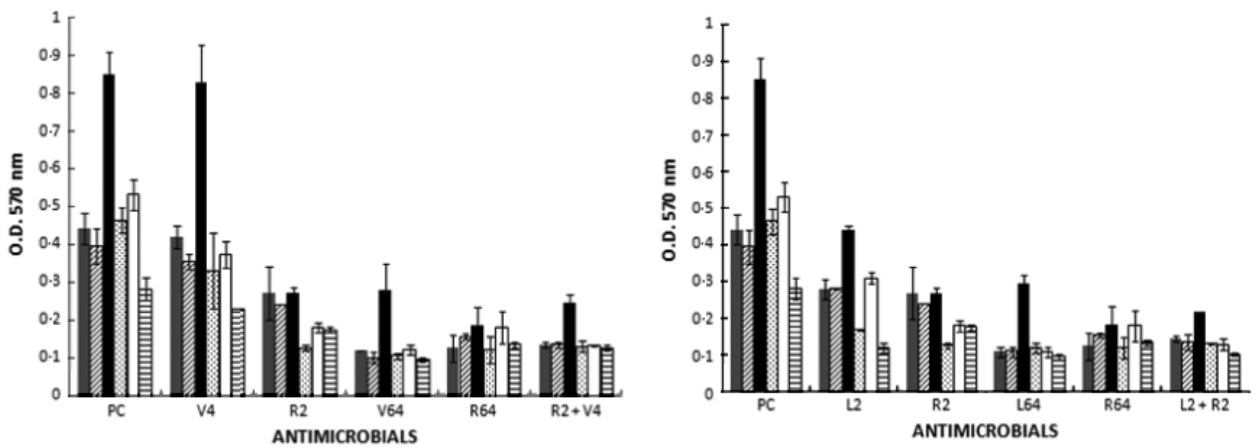


**Figure 4** Biomass reduction in six MRSA-SCC*mec* IV isolates exposed to different concentrations of antimicrobial agents applied individually or combined, in relation to the positive control (PC) without antimicrobial exposure. $V_4$–4 $\mu g\ ml^{-1}$ of vancomycin, $V_{64}$–64 $\mu g\ ml^{-1}$ of vancomycin, $R_2$–2 $\mu g\ ml^{-1}$ of rifampicin, $R_{64}$–64 $\mu g\ ml^{-1}$ of rifampicin, $L_2$–2 $\mu g\ ml^{-1}$ of linezolid, $L_{64}$–64 $\mu g\ ml^{-1}$ of linezolid. (■) 633 (USA400/ST1); (▨) 915 (US400/ST1); (■) 1177 (USA800/ST5); (▦) 1112 (USA800/ST5); (□) 943 (USA1100/ST30) and (▤)1314 (USA1100/ST30).

the drug. However, no significant biomass reduction was observed, even in concentrations of vancomycin >20 $\mu g\ ml^{-1}$. According to Wells *et al.* (2011), some factors may contribute to differences in the antimicrobial efficacy against biofilms/biomass, such as differences in the penetration levels of the antimicrobials into the biofilm/biomass, reduced bacterial growth rate and/or increased expression of resistance genes.

In the present study, the synergic activity of antimicrobials was more effective in reducing both biomass and number of viable cells than the drugs alone. Rifampicin (2 $\mu g\ ml^{-1}$) *plus* linezolid (2 $\mu g\ ml^{-1}$) was one of the most effective combinations, demonstrating an enhanced antibacterial effect when compared to monotherapy. According to a previous study, rifampicin is a constituent of all the combinations that are active against MRSA and is included in antibiotic therapy directed against biofilms/biomass formed by these organisms (Saginur *et al.* 2006). When the combination effect of oral antibiotics was evaluated for 33 biofilm-embedded MRSA isolates, rifampicin

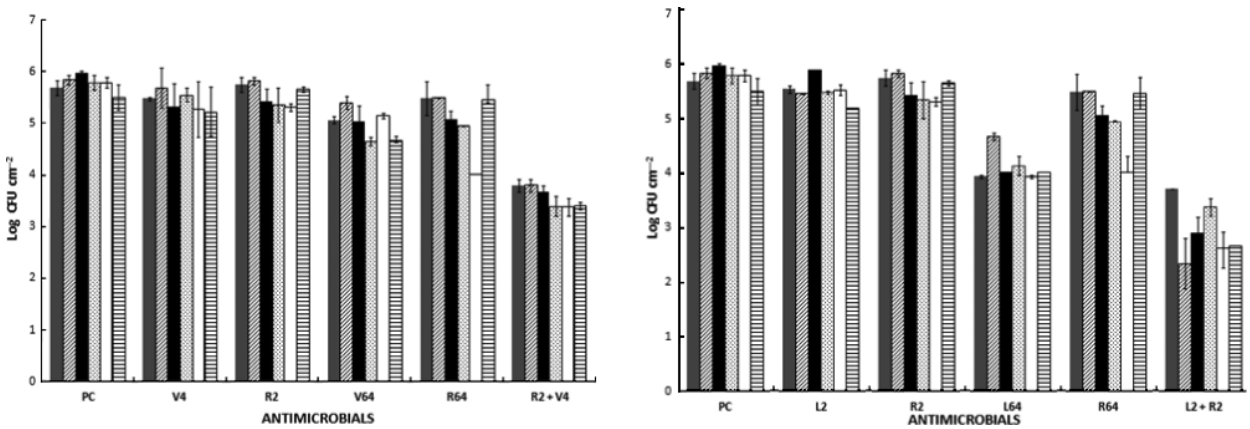


**Figure 5** Cell viability (CFU $cm^{-2}$) reduction in six MRSA-SCC*mec* IV isolates exposed to different concentrations of antimicrobial agents applied individually or combined, in relation to the positive control (PC) without antimicrobial exposure. $V_4$–4 $\mu$g $ml^{-1}$ of vancomycin, $V_{64}$–64 $\mu$g $ml^{-1}$ of vancomycin, $R_2$–2 $\mu$g $ml^{-1}$ of rifampicin, $R_{64}$–64 $\mu$g $ml^{-1}$ of rifampicin, $L_2$–2 $\mu$g $ml^{-1}$ of linezolid, $L_{64}$–64 $\mu$g $ml^{-1}$ of linezolid. (■) 633 (USA400/ST1); (▨) 915 (US400/ST1); (■) 1177 (USA800/ST5); (▩) 1112 (USA800/ST5); (□) 943 (USA1100/ST30) and (▤)1314 (USA1100/ST30).

plus linezolid had a better synergism than other antimicrobial combinations (Wu *et al.* 2012), which was verified in the present study. Raad *et al.* (2007) demonstrated that rifampicin was highly active when associated with other antibiotics, particularly linezolid, in eradicating MRSA colonization on silicone discs. Also, Vergidis *et al.* (2011) demonstrated that combination treatment with linezolid plus rifampin or vancomycin plus rifampin was effective in an animal model of MRSA foreign body osteomyelitis in the context of retention of the infected foreign body. Although no significant differences have been found, this antimicrobial combination presented *P* values close to 0·05 in relation to the reduction in biomass and to the viable cell number when compared with other drug combinations. However, the reduced number of MRSA isolates evaluated may have led to these findings, and thus, further investigations involving more isolates are necessary to confirm these results.

In our study, another synergism that was observed to present a good activity against MRSA isolates was rifampicin (2 $\mu$g $ml^{-1}$) plus vancomycin (4 $\mu$g $ml^{-1}$). Reiter *et al.* (2012) studied the activity of rifampicin, individually and in combination with vancomycin, against biofilm producer isolates of MRSA. They found greater inhibition of bacterial growth after combined drug use than after use of each drug individually. However, the authors commented that the noneradication of the biofilm/biomass might contribute to bacterial persistence. Silva *et al.* (2011) evaluated the synergic potential of subminimum inhibitory concentrations of rifampicin plus vancomycin against clinical isolates of MRSA and coagulase-negative *Staphylococcus*. However, the authors observed a satisfactory synergistic effect in only two and three of the 22 isolates that were evaluated, respectively.

In the present study, the combination between linezolid and vancomycin against staphylococci biofilm/biomass was not satisfactory, especially the ineffective reduction in cell viability. Singh *et al.* (2009) observed that no synergistic activity was seen when these two antimicrobial agents were combined, *in vitro*, against five MRSA isolates from bloodstream infections.

Microscopic analysis showed similar results to the *in vitro* susceptibility tests, that is, only after exposure to drug combinations, a decrease in the number of viable cells was verified, as already observed by other authors (Cha *et al.* 2011).

Schuenck *et al.* (2012) showed that all isolates of MRSA were positive for the presence of the *ica*A gene, confirming the findings of the present study. The *sas*G gene was investigated by Rasmussen *et al.* (2013) who found a correlation between invasive *Staph. aureus* isolates and the presence of this gene. It is possible that the polysaccharide intercellular adhesin (PIA), essential substance in biofilm adhesion step, as well as, surface proteins of staphylococci, such as SasG present in *ica*-negative staphylococcal biofilms (Geoghegan *et al.* 2010), have some participation in biomass formation and thus contribute to the connection between cells on the material surface.

In summary, this study showed that MRSA-SCC*mec* IV isolates are, in general, weak or nonbiofilm producers and lineages belonging to STs 1, 5 and 30, irrespective of biofilm production, presented biomass reduction after exposure to different antimicrobials. Furthermore, antimicrobial synergisms involving $Rif_{2\mu g\,ml^{-1}} + Lin_{2\mu g\,ml^{-1}}$ and $Rif_{2\mu g\,ml^{-1}} + Van_{4\mu g\,ml^{-1}}$ appear to be good therapy choices, as both combinations produced greater reductions in biomass and the number of viable staphylococcal

cells. Therefore, these synergistic drug combinations might be considered in the chemotherapy of MRSA-SCC*mec* IV infections.

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## Conflict of interest

The authors declare there are no conflict of interests.

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