

## ORIGINAL PAPER

# Action of antibiotic oxacillin on *in vitro* growth of methicillin-resistant *Staphylococcus aureus* (MRSA) previously treated with homeopathic medicines



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**Background:** Resistance to antibiotics is a major public health concern worldwide. New treatment options are needed and homeopathy is one such option. We sought to assess the effect of the homeopathic medicine *Belladonna* (Bell) and a nosode (biotherapy) prepared from a multi-drug resistant bacterial species, methicillin-resistant *Staphylococcus aureus* (MRSA), on the same bacterium.

**Methods:** Bell and MRSA nosode were prepared in 6cH and 30cH potencies in 30% alcohol and sterile water, according to the Brazilian Homeopathic Pharmacopeia and tested on MRSA National Collection of Type Cultures (NCTC) 10442. We assessed *in vitro* bacterial growth, deoxyribonuclease (DNAase) and hemolysin activity, and *in vitro* bacterial growth in combination with oxacillin (minimum inhibitory concentration – MIC). All values were compared to control: 30% alcohol and water.

**Results:** *In vitro* growth of MRSA was statistically significantly inhibited in the presence of Bell and nosode 6cH and 30cH compared to controls ( $p < 0.0001$ ); and with combination of Bell or nosode 6cH and 30cH and oxacillin ( $p < 0.001$ ). Bell 30cH and nosode 6cH and 30cH significantly decreased bacterial DNAase production ( $p < 0.001$ ) and reduced red blood cell lysis.

**Conclusions:** Cultures of MRSA treated with *Belladonna* or MRSA nosode exhibited reduced growth *in vitro*, reduced enzymatic activity and became more vulnerable to the action of the antibiotic oxacillin. Further studies are needed on the biomolecular basis of these effects. *Homeopathy* (2017) 106, 27–31.

**Keywords:** MRSA; *Belladonna*; Nosode; Oxacillin; *In vitro* growth; DNase; Hemolysin

## Introduction

Resistance to antibiotics is a major public health concern worldwide; growing resistance to multiple drugs is attributed to excessive use of antimicrobials.<sup>1</sup> According to the World Health Organization, infectious diseases are the third commonest cause of death around the world, in spite of antibiotic therapy.<sup>2</sup> This situation is exacerbated by the increased incidence of infections with multidrug-resistant

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bacterial strains, whence the need for new treatment options able to eliminate pathogens and avoid development of drug resistance.

One such option is homeopathy.<sup>3–5</sup> Recent studies demonstrate that homeopathic treatment might improve the patients' clinical condition, reduce the need for conventional antimicrobial agents and decrease the rate of infection relapse.<sup>6</sup> Some authors suggested that homeopathic medicines might have antimicrobial effects.<sup>4,7</sup> Thus we started a research project to evaluate the *in vitro* effect of homeopathic drugs on bacterial cultures. Our first study showed that *Belladonna* 12cH and 30cH and *Streptococcus pyogenes* nosode 12cH and 30cH inhibited the *in vitro* growth of *S. pyogenes*, thus pointing to a direct action of the tested drugs on the bacteria.<sup>8</sup>

In the present study we sought to assess the effect of *Bell* and an isopathic nosode on a multi-drug resistant bacterial species, methicillin-resistant *Staphylococcus aureus* (MRSA), which became a serious health problem in the past 15 years.<sup>9</sup> In addition, we also sought to evaluate potential synergism between homeopathic and conventional antibiotic treatment. We measured the minimum inhibitory concentration (MIC) of oxacillin in MRSA cultures previously incubated with these homeopathic medicines. In Brazil, oxacillin is used instead of methicillin; the two drugs are similar and exhibit the same mechanism of bacterial resistance.<sup>10</sup>

## Materials and methods

### Preparation of the bacterial culture

We used MRSA NCTC (National Collection of Type Cultures) 10,442 grown in agar-blood medium and incubated at 37°C for 20 h. Next a bacterial suspension in sterile saline solution measuring 0.5 on the McFarland scale was prepared. The suspension was also diluted in Mueller–Hinton (M–H) broth (Difco, USA) 1:10 before use.

### Homeopathic medicines

The homeopathic medicines were prepared according to the 3rd edition of the Brazilian Homeopathic Pharmacopoeia.<sup>11</sup> *Bell* and MRSA nosode were diluted and agitated in 30% ethanol or sterile water to potencies 6cH and 30cH (HN-Cristiano, Brazil).

### Test tube trials

A 250- $\mu$ L volume of each tested homeopathic medicine was added to tubes containing 3 mL of M–H broth. After homogenization, 10  $\mu$ L of bacterial suspension diluted 1:10 in sterile saline were added to each tube. The tubes were homogenized and incubated at 37°C for 24 h. After that, bacterial growth was evaluated with spectrophotometer (Gehaka UV-330G, Brazil) at 600 nm.<sup>12</sup> The effect of 30% ethanol or water (control) on bacterial growth was assessed under the same conditions as with the homeopathic medicines. Sterility was tested by sowing 250  $\mu$ L of each drug in M–H broth. Three independent experiments were done in quadruplicate.

### MIC of oxacillin in the presence of homeopathic medicines

MIC of oxacillin was determined according to CLSI (Clinical and Laboratory Standards Institute) 2014 standards.<sup>13</sup> A volume of 420  $\mu$ L of *Bell* or *S. aureus* nosode 6cH and 30cH was added to 5 mL of cation-adjusted M–H broth (M–H II). A volume of 20  $\mu$ L of MRSA suspension measuring 0.5 on the McFarland scale and diluted 1:10 in sterile saline was added to the tubes. The tubes were incubated at 37°C for 3 h. A 50- $\mu$ L volume of the bacterial suspensions was added to 96-well flat-bottom plates, previously prepared with 50  $\mu$ L of serial dilutions of oxacillin in M–H II broth in concentrations ranging from 16 to 0.5  $\mu$ g/mL. The plates were incubated at 37°C for 24 h, and bacterial growth was measured with spectrophotometer at 600 nm.<sup>12</sup> The effect of 30% ethanol or water (control) on bacterial growth was assessed under the same conditions as with the medicines. All the tests were performed in quadruplicate. According to CLSI 2014 criteria the MIC of oxacillin for MRSA is 4  $\mu$ g/mL.<sup>13</sup> Three independent experiments were done in quadruplicate.

### Enzymatic activity

The activity of *S. aureus* enzymes deoxyribonuclease (DNase) and hemolysin were analyzed by means of DNase Test Agar (BD, USA) and red blood cell lysis in Agar blood (BD, USA) respectively. MRSA diluted to 0.5 on the McFarland scale were treated with *Bell* or nosode 6cH and 30cH prepared in 30% alcohol or water at 37°C for 20 h. The results were expressed as diameter of the bacterial growth inhibition zone and were compared between treated groups and controls (30% alcohol and water). Three independent experiments were done in quadruplicate.

### Statistical analysis

The experimental data were evaluated by means of analysis of variance (ANOVA) and Tukey's multiple comparison test with standard deviation and significance level  $p < 0.05$  (Graphpad software Prism version 5).

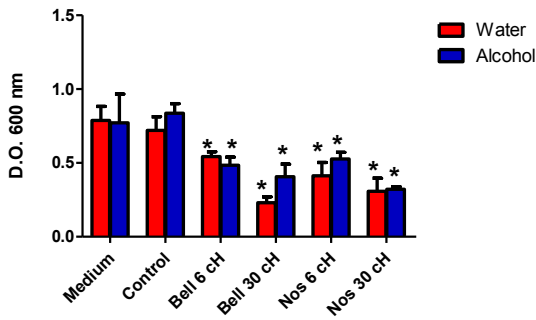
## Results

### Test tube experiments

*In vitro* growth of MRSA exhibited statistically significant reduction in the presence of *Bell* and nosode 6cH and 30cH compared to controls – 30% alcohol and water ( $p < 0.0001$ ) The results demonstrated significant decrease in bacterial growth after treatment with *Bell* or nosode 6cH and 30cH, independently whether prepared with 30% alcohol or water (Figure 1).

### MIC of oxacillin in the presence of homeopathic medicines

To calculate the MIC of oxacillin for MRSA we first evaluated the growth of bacteria treated with 30% alcohol or water. This analysis showed that neither 30% alcohol or water not induced significant changes in the *in vitro* growth

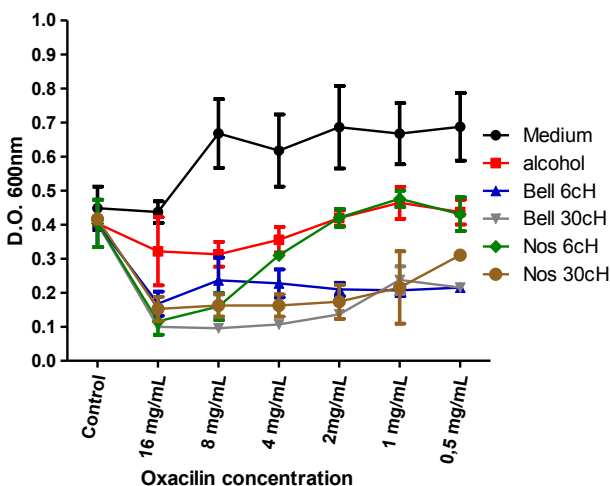


**Figure 1** Effect of homeopathic medicines (Bell: *Belladonna*; Nos: MRSA nosode) on MRSA *in vitro* growth. Bacteria were cultured with (medium M–H and vehicle water or 30% alcohol) or with *Bell* or Nos 6cH and 30cH prepared with water or 30% alcohol. Statistically significant differences were investigated between controls and medicines treated bacteria (\* $p < 0,001$  ANOVA and Tukey). The tests were done N = 12.

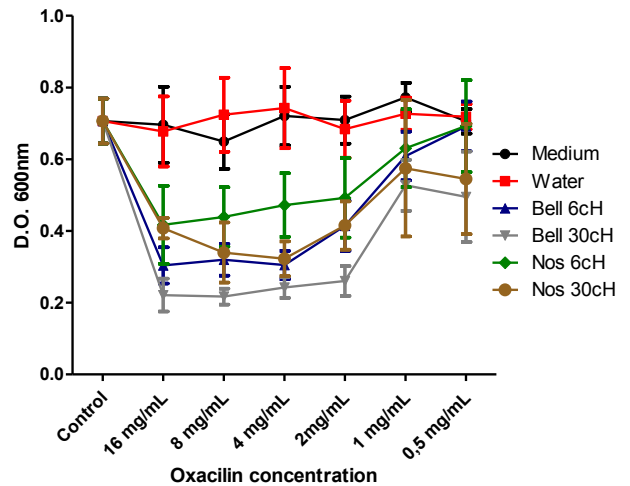
of the bacteria in the presence of oxacillin, and that the growth rate was similar to the one of the bacteria cultured in M–H II broth (Figures 2 and 3). The tests were done N = 12 with ANOVA and Tukey statistical analysis.

None of the tested homeopathic medicines and potencies induced total inhibition of the bacterial growth following treatment with oxacillin. Nevertheless, *in vitro* growth of MRSA first treated with *Bell* 6cH or 30cH prepared with 30% alcohol and then incubated with oxacillin showed significant decrease with all the antibiotic concentrations ( $p < 0.0001$ ). The percent inhibition of bacterial growth was greater with the highest concentrations of antibiotic (Figure 2).

Also *in vitro* growth of MRSA first treated with nosode 6cH or 30cH prepared with 30% alcohol and then incubated with oxacillin showed statistically significant changes. Combination of nosode 6cH and oxacillin in con-



**Figure 2** Effect of homeopathic medicines (Bell: *Belladonna*; Nos: MRSA nosode) combined with oxacillin on MRSA *in vitro* growth. Bacteria were cultured with 30% alcohol (control) or with *Bell* or Nos 6cH and 30cH diluted in 30% alcohol and combined with oxacillin 16, 8, 2, 1 and 0.5 mg/mL. Statistically significant differences were investigated between control oxacillin alone or in combination with *Bell* 6cH at concentrations of 8, 4, 2, 1, and 0.5 mg/mL ( $p < 0,0001$  ANOVA and Tukey). The tests were done N = 12.



**Figure 3** Effect of homeopathic medicines (Bell: *Belladonna*; Nos: MRSA nosode) combined with oxacillin on MRSA *in vitro* growth. Bacteria were cultured with sterile water (control) or with *Bell* or Nos 6cH and 30cH diluted in sterile water and combined with oxacillin 16, 8, 2, 1 and 0.5 mg/mL. Statistically significant differences were investigated between control or medicine treated MRSA alone or in combination with oxacillin at 16, 8, 4, 2, 1, and 0.5 mg/mL ( $p < 0,0001$  ANOVA and Tukey). The tests were done N = 12.

centrations 16, 8 and 4  $\mu\text{g/mL}$  induced reduction of the bacterial growth ( $p < 0.05$ ). In turn, combination of nosode 30cH and oxacillin in concentrations 16, 8, 4, 2 and 1 mg/mL reduced MRSA *in vitro* growth ( $p < 0.0001$ ). The curve that represents the results obtained with the various antibiotic concentrations shows that the greater the concentration, the higher the inhibition of bacterial growth (Figure 2).

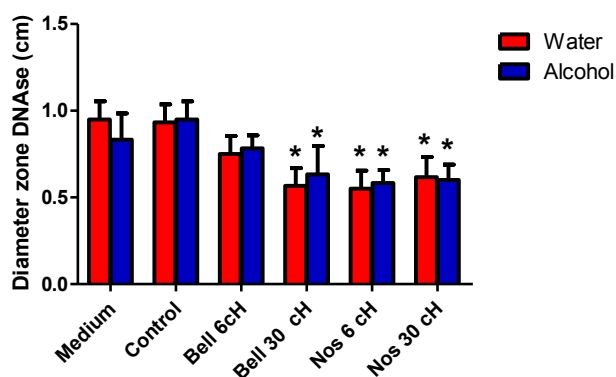
Treatment of MRSA with combination of *Bell* 6cH or 30cH prepared with water and 16, 8, 4 and 2 mg/mL of oxacillin induced significant decrease of bacterial growth ( $p < 0.0001$ ) (Figure 3). Similarly, treatment with combination of nosode 30cH and oxacillin 8 and 4 mg/mL significantly decreased MRSA growth ( $p < 0.001$ ) (Figure 3). In turn, treatment with nosode 6cH (prepared with water) combined with oxacillin did not inhibit bacterial growth *in vitro* (Figure 3).

### Enzymatic activity

Treatment of MRSA with *Bell* 6cH prepared with 30% alcohol or water did not induce changes in DNase activity compared to the controls (30% alcohol or water). In turn, treatment of MRSA with *Bell* 30cH and nosode 6 or 30cH prepared with 30% alcohol or water significantly decreased the bacterial DNase production ( $p < 0.001$ ) (Figure 4). Similarly, *Bell* and nosode 6 and 30cH treatment reduced red blood cell lysis in agar blood (Table 1).

## Discussion

Bacterial resistance to antibiotics is a serious health problem worldwide.<sup>1</sup> Homeopathy has been studied as possible treatment for infections with drug-resistant microorganisms. In one study, patients with tuberculosis caused



**Figure 4** *Staphylococcus aureus* deoxyribonuclease production determined by diameter (cm) of DNA hydrolysis area with medium, water or 30% alcohol (control), and treatment with *Belladonna* (Bell) or MRSA nosode (Nos) 6cH and 30cH diluted in water or 30% alcohol. Statistically significant differences between controls or medicines treated MRSA nosode (\* $p < 0,001$  ANOVA and Tukey). The tests were done  $N = 12$ .

by *Mycobacterium tuberculosis* resistant to rifampicin and isoniazid were treated with the standard regime combined with individualized homeopathy or placebo. The results showed statistically significant improvement of all outcome measures, including clinical status and radiological signs, except for sputum culture conversion.<sup>5</sup> Homeopathy was tested as an alternative to antibiotics in livestock production systems. In one study, the incidence of *Escherichia coli* diarrhea was significantly lower in the offspring of sows treated with *E. coli* nosode 30K compared to placebo.<sup>14</sup> Ultra-diluted and agitated solutions also act upon live cells changing their gene expression and biological activity.<sup>15,16</sup> These findings demonstrate effective biological action of homeopathic medicines on cells leading to changes in their physiology.<sup>15–17</sup>

In our first study we investigated the action of homeopathic medicines on *S. pyogenes* cultures. The results showed significant growth reduction in the cultures incubated with *Bell* or *S. pyogenes* nosode.<sup>8</sup> In the present study, we investigated the effects of *Bell* and bacterial nosode on cultures of MRSA, a multi-drug resistant pathogen and a major cause of hospital-acquired infections.<sup>1,18</sup> The only mention to the effect of homeopathic treatment on MRSA we could locate in the scientific literature is a report of a successfully treated case of skin abscess.<sup>19</sup>

**Table 1** MRSA hemolysin enzymatic activity after treatment with medium, water or 30% alcohol (control) and with *Belladonna* (Bell) or nosode (Nos) 6cH and 30cH diluted in water or 30% alcohol

	Water	Alcohol
Medium	+++	+++
Control	+++	+++
<i>Bell</i> 6cH	++	++
<i>Bell</i> 30cH	+	+
Nos 6cH	+	++
Nos 30cH	+	+

+++ : 100% red blood cell lysis; ++ : 70% red blood cell lysis; + : 40% red blood cell lysis.

*Atropa belladonna* L., the source of homeopathic medicine *Bell*, is a perennial woodland herbaceous plant that belongs to family Solanaceae and exhibits high toxicity.<sup>20</sup> The pathogenetic picture of *Bell* includes feeling of heat, redness, aggressiveness, irritability and fever.<sup>21,22</sup> *S. aureus* is associated with skin infections such as painful boils or abscesses characterized by presence of pus, redness, local heat and fever in severely ill patients.<sup>23</sup> The similarity between the natural infection and *Bell* pathogenetic effects justifies the choice of this homeopathic drug in the present study.

The results showed that *Bell* 6cH and 30cH induced significant decrease of the bacterial growth rate of about 40–50%. To control for possible non-specific effects, we used *Bell* prepared in 30% alcohol (standard preparation) and water and compared it to the corresponding controls; the effect was obtained in both cases without significant difference. Here we also have a strong basis to rule out non-specific effects of diluted and agitated alcohol.

In the present study we also performed simple tests of enzymatic activity (DNase and hemolysin) to evaluate other aspects of *S. aureus* biology. The results showed that *Bell* 6cH prepared with either 30% of alcohol or water did not induce changes in DNase activity, but reduced red blood cell lysis. In turn *Bell* 30cH prepared with either 30% alcohol or water significantly reduced DNase activity and red blood cell lysis.

Taken all together our results indicate that homeopathic drug *Belladonna* induces negative effects on MRSA growth and some biological parameters, which might somehow contribute to its indication for treatment of bacterial infections provided the patient's and the remedy's symptoms are similar. As to the lack of effect of *Bell* 6cH on DNase activity, we were not able to locate any data in the literature to account for it. While one might suspect that it might be somehow related with the fact that potency 6cH is below Avogadro's number, this hypothesis is inconsistent with the results on the red blood cell lysis test and data on gene regulation published in the literature.<sup>24,25</sup> As a whole, the mechanisms by which the homeopathic medicines acted on the bacteria are unknown, and further research is needed to explain them, including analysis of the gene and protein expression of molecules involved in the synthesis of bacterial cell wall, deoxyribose and hemolysin, as well as of staphylococcal cassette chromosome *mec* elements (*SCCmec*).<sup>9,26</sup>

The use of nosodes is based on the isopathic principle, according to which a disease can be cured by the same agent that causes it,<sup>27</sup> which means that highly diluted and agitated solutions of a pathogen may influence infections caused by the same pathogen species.<sup>14,28</sup>

In the present study, MRSA nosode 6cH and 30cH induced 40–60% reduction of *in vitro* bacterial growth compared to the controls treated with 30% alcohol or water. In addition, the nosode reduced *S. aureus* DNase and hemolysin activity with both tested potencies and pharmaceutical vehicles used.

In the second step of the study, MRSA treated with *Bell* or nosode was incubated with different concentrations of oxacillin. The results showed that the cultures became once again susceptible to the action of the antibiotic, as their growth exhibited 60–80% inhibition compared to the controls treated with 30% alcohol or water. That reduction was greater compared to the one induced by the homeopathic medicines alone. This phenomenon was not due to bacterial cell death, but to sharp decrease in their growth speed, which probably made the bacteria become more sensitive to oxacillin.

## Conclusion

Cultures of the MRSA treated with *Belladonna* or nosode exhibited reduced growth *in vitro*, reduced enzymatic activity and became more vulnerable to the action of antibiotic oxacillin. Further studies are needed to account for the biomolecular grounds of these effects.

## Conflict of interest

None.

## Funding sources

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