

Short Communication

Action of Some Homeopathic Medicine on the Growth of Methicillin-Resistant *Staphylococcus aureus* (MRSA) *In vitro*

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Submitted: 16 June 2017

Accepted: 10 August 2017

Published: 17 August 2017

ISSN: 2475-9430

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OPEN ACCESS**Keywords**

- MRSA
- Belladonna
- Nosode
- Oxacillin
- In vitro growth

Abstract

Resistance to antibiotics became a major public health concern worldwide, whence new treatment are needed and homeopathy medicines used in pyogenic infection is one the options. In the present study we aimed to assess the effect of homeopathic medicines *Silicea*, *Hepar sulfur*, *Belladonna* and bacterial nosode on a MRSA. The medicine were prepared in potencies 6 CH, 12 CH and 30 CH in sterile 30% alcohol and tested on MRSA NCTC 10442; the following parameters were assessed: *in vitro* bacterial growth and *in vitro* bacterial growth in combination with oxacillin MIC; and all the tests were compared to controls 30% alcohol. *In vitro* growth of MRSA exhibited statistically significant reduction in the presence of *Belladonna*, nosode 6 CH and 30 CH, *Hepar sulfur* 30 CH and *Silicea* 6 CH compared to 30% alcohol ($p < 0.0001$). The combination of *Belladonna* or nosode 6 CH and 30 CH and oxacillin showed a statistically significant reduction ($p < 0.001$). However the combination of *Silicea* or *Hepar sulfur* and oxacillin did not show statistically significant reduction. It can therefore be concluded that cultures of the MRSA treated with homeopathy respond differently to different homeopathic medicines. The results demonstrate that live cells respond in a particular way for different sorts of medicines and different potencies.

ABBREVIATIONS

NCTC: National Collection of Type Cultures; MIC: Minimum Inhibitory Concentration; *Bell*: Belladonna; *Nos*: Nosod

INTRODUCTION

Staphylococcus aureus developed resistance to methicillin. Methicillin-resistant *Staphylococcus aureus* (MRSA) is actually resistant to an entire class of penicillin-like antibiotics called beta-lactams [1]. Recent studies demonstrate that homeopathic treatment might improve the patients' clinical condition, reducing the need for conventional antimicrobial agents and decreasing the rate of infection relapse [2,3]. Authors suggested that homeopathic medicines might have antimicrobial effects [4,5]. Therefore, we started a research project to evaluate the *in vitro* effect of homeopathic drugs used in pyogenic infections [6-8]. Our study showed that *Belladonna* 6 CH and 30 CH and MRSA nosode 6 CH and 30 CH inhibited the *in vitro* growth of MRSA, while the combination of *Belladonna* or nosode 6 CH and 30 CH and oxacillin increased the antibiotic effect on bacterial growth [9]. In the present study we aimed to compare the effect of *Belladonna*, isopathic nosode on MRSA, *Silicea* and *Hepar sulfur* on bacterial growth *in vitro*. 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 [10].

MATERIALS AND METHODS

We used MRSA NCTC (National Collection of Type Cultures) 10,442 grown in agar-blood medium and incubated at 37°C for 20 h. Then a bacterial suspension in sterile saline solution measuring 0.5 on the McFarland scale was prepared. The suspension was also diluted in Mueller Hinton (MH) broth (Difco, USA) 1:10 before use. The homeopathic medicines were prepared according to the 3rd edition of the Brazilian Homeopathic Pharmacopoeia [11]. The medicine were diluted and dynamized in 30% alcohol to potencies 6 CH, 12 CH and 30 CH (HN-Cristiano, Brazil). A 250 µL volume of each tested homeopathic medicine was added to tubes containing 3 mL of MH broth. Then 10 µL of bacterial diluted 1:10 was 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 [12]. MIC of oxacillin in the presence of homeopathic medicines was determined according to CLSI (Clinical and Laboratory Standards Institute) 2014 standards [13]. A volume

of 420 μL of *Belladonna* or MRSA nosode 6 CH and 30 CH or *Silicea* 6 CH or *Hepar sulfur* 30 CH, was added to 5 mL of cation-adjusted MH broth (MH II). A volume of 20 μL of MRSA in the same diluted. The tubes were incubated at 37°C for 3 h. A 50 μL volume of the bacterial suspensions was added to 96-multiwell plates, previously prepared with 50 μL of serial dilutions of oxacillin in MH II broth in concentrations ranging from 16 to 0.5 mg/mL. The plates were incubated at 37°C for 24 h, and bacterial growth was measured with spectrophotometer at 600 nm¹². According to CLSI 2014 criteria the MIC of oxacillin for MRSA is 4 mg/mL [13]. The experimental data were evaluated by analysis of variance (ANOVA) and Tukey's test with significance level of $p < 0.05$ (Graphpad software Prism version 5).

RESULTS AND DISCUSSION

Passeti 2016, evidenced the *in vitro* growth of MRSA exhibited statistically significant reduction in the presence of *Bell* and nosode 6 CH and 30 CH. Treatment of MRSA with combination of *Bell* 6 CH and 30 CH or nosode 30CH and 16, 8, 4 and 2 mg/mL of oxacillin induced significant decrease of bacterial growth. The results showed that combination *Bell* or nosode with antibiotic induced increased inhibition of the bacterial growth than homeopathic medicine alone [9]. In this study we compared the effect of *Silicea* and *Hepar sulfur* medicines on the *in vitro* growth of MRSA bacterial, knowing that these medicines are also used for pyogenic bacterial infections.

The results demonstrate that *Silicea* 6 CH ($p < 0.001$) and

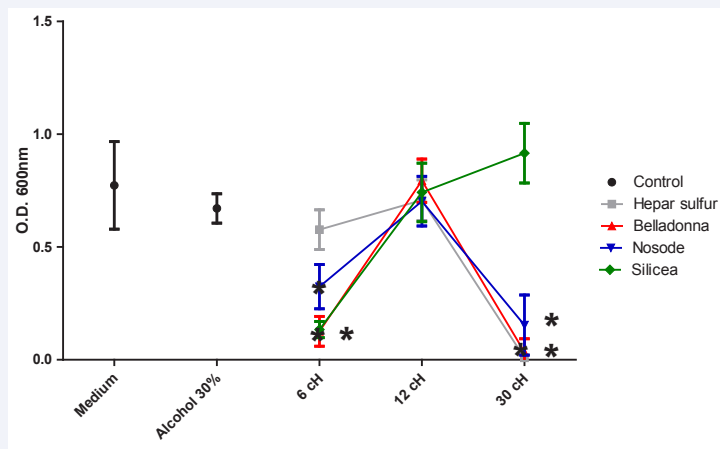


Figure 1 Effect of homeopathic medicines on MRSA *in vitro* growth. Bacteria were cultured with (MH medium and 30% alcohol) or with *Bell*, *Nos*, *Silicea* and *Hepar sulfur* 6CH, 12CH and 30CH prepared with 30% alcohol. Statistically significant differences were investigated among 30% alcohol and medicines treated bacteria (* $p < 0.001$ ANOVA and Tukey).

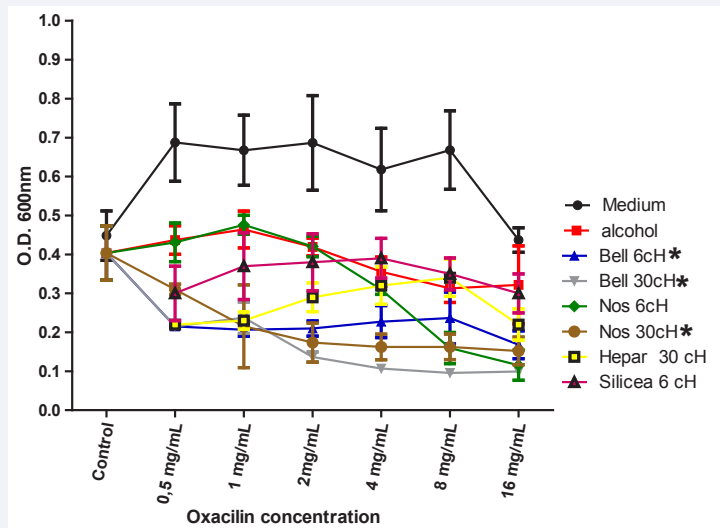


Figure 2 Effect of homeopathic medicines combined with oxacillin on MRSA *in vitro* growth bacteria were cultured (control with 30% alcohol) or with *Bell* or *Nos* 6CH and 30CH, *Hepar sulfur* 30CH or *Silicea* 6CH diluted in 30% alcohol and combined with oxacillin 16, 8, 2, 1 and 0.5 mg/mL. Statistically significant differences were investigated among control or medicine treated MRSA alone or in combination with oxacillin at 4 mg/mL (MIC) (* $p < 0.0001$ ANOVA and Tukey).

Abbreviations: Bell: Belladonna; Nos: MRSA nosode; Hepar: Hepar sulfur

Hepar sulfur 30 CH ($p < 0,001$) can inhibit the *in vitro* MRSA growth (Figure 1). The combination of these medicines with oxacillin antibiotic did not show influence on the *in vitro* bacterial growth (Figure 2). We suggest that only *Belladonna* and the MRSA nosode have the ability to make the bacterial more susceptible to the antibiotic action.

Assessing the activity of the different potencies on *in vitro* bacterial growth, we observed that 12 CH potency did not show action on the cultures. This demonstrates that living cells respond differently to different homeopathic potencies, than suggesting that a further kinetic study may be appropriate.

CONCLUSION

Belladonna or nosode interfere in the metabolism of the MRSA and alter it to become more susceptible to the oxacillin antibiotic action in bacterial culture. The MRSA treated with *Silicea* and *Hepar sulfur* reduced *in vitro* growth, but didn't interfere on oxacillin antibiotic action. Different potencies of the same medicine produce different responses on the micro-organism growth, suggesting that a further kinetic study may be appropriate.

ACKNOWLEDGEMENTS

Funding Sources: ABC Medical School of Santo André Foundation

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Cite this article

Passeti TA, de Andrade Ruggero A, Bissoli LR, da Silva RNC, Affonso Fonseca FL (2017) Action of Some Homeopathic Medicine on the Growth of Methicillin-Resistant *Staphylococcus aureus* (MRSA) *In vitro*. *Ann Clin Cytol Pathol* 3(6): 1074.