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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 DNAse 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.[1] According to the World Health Organization, infectious diseases are the third commonest cause of death around the world, in spite of antibiotic therapy.[2] 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.[3–5] 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.[6] Some authors suggested that homeopathic medicines might have antimicrobial effects.[4,7] 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.[8]

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.[9] 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.[10]

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.[11] *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-μL volume of each tested homeopathic medicine was added to tubes containing 3 mL of M–H broth. After homogenization, 10 μl 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.[12] 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 μL 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.[13] A volume of 420 μL 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 μL 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-μL volume of the bacterial suspensions was added to 96-well flat-bottom plates, previously prepared with 50 μL of serial dilutions of oxacillin in M–H II broth in concentrations ranging from 16 to 0.5 μg/mL. The plates were incubated at 37°C for 24 h, and bacterial growth was measured with spectrophotometer at 600 nm.[12] 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 μg/mL.[13] 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