LOOKING FOR NEW COMPOUNDS TO BATTLE ANTIBIOTIC RESISTANCE: OPTIMIZATION OF ORGANIC SOLVENTS

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Abstract

A high number of pathogens have shown the capacity of resist to the action of antibiotics. This fact presents a very important problem to public health and highlights the necessity of looking for new substances as alternative to antibiotics.

Since some of these substances have a hydrophobic nature they need to be solved in organic solvents. Some of these solvents are also toxic to bacteria, mainly affecting membranes. For these reasons it is necessary to perform a study of the toxicity of organic solvents to bacteria, which has been the aim of this work.

Five organic solvents, DMF (dimethylformamide), TBME (tert-butyl methyl ether), THF (tetrahydrofuran), DMSO (dimethyl sulfoxide) and CH2Cl2 (dichloromethane), have been tested, using two of the most prevalent pathogens (*Staphylococcus pseudintermedius* and *Pseudomonas aeruginosa*,) both in animals and humans.

The results show that, when possible, DMSO followed by TBME are the best options for testing new compounds in *Staphylococcus pseudintermedius*, whether DMF followed by TBME are the preferred solvents for testing hydrophobic compounds in *Pseudomonas aeruginosa*.

1. Introduction and objectives of the study

Due to the emerging problem of antibiotics resistance, looking for new compounds to inhibit bacterial growth has become indispensable (Somayaji *et al.*, 2016; Fungwithaya *et al.*, 2017; Vingopouloua *et al.*, 2018). In order to test a big number of compounds with different chemical properties it is necessary the use of organic solvents (Galvao *et al.*, 2014; Radošević *et al.*, 2018). The main objective of the present study is to determine the toxicity rate and impact of these organic solvents in bacterial cultures of *Staphylococcus pseudintermedius* and *Pseudomonas aeruginosa*, two of the most frequent multiresistant pathogens in small animals and potential zoonoses (Somayaji *et al.*, 2016; Fungwithaya *et al.*, 2017; Vingopouloua *et al.*, 2018). This study lays the foundations for further investigation using polymers and nanoparticles for the same purpose.

2. Material and Methods

Two bacterial strains have been tested, *Staphylococcus pseudintermedius* from the Belgium Collection of Bacterial Cultures (LMG22219) and *Pseudomonas aeruginosa* from the Spanish Collection of Bacterial Cultures (CECT 110).

The essay includes five organic solvents: DMF (Dimethylformamide), TBME (*tert*-butyl methyl ether), THF (Tetrahydrofuran), DMSO (Dimethyl sulfoxide) and CH_2Cl_2 (dichloromethane).

Three serial dilutions of liquid bacterial cultures (TSB) per solvent were used:

- 1/1000 dilution: 1ml of TSB and 1µl of pure solvent.
- 1/100 dilution: 1ml of TSB and 10µl of pure solvent.
- 1/10 dilution: 900µl of TSB and 100µl of pure solvent.

As controls, we use TSB medium alone (negative control) and TSB medium inoculated (positive control). A total of 34 tubes were tested, 17 for each strain (3 dilutions x 5 repetitions, together with two positive and negative controls). Once the dilutions were prepared, they were incubated for 24 hours at 37 °C. After that, the presence of bacterial growth was evaluated using both qualitative and quantitative methods. As a qualitative indicator, the turbidity of the dilution was used; the more turbid the medium is, the more bacterial growth is present. As quantitative method the absorbance at 600 nm of wavelength was measured, obtaining a turbidity value, which is directly proportional to bacterial growth.

3. Results

An easy and quick visual evaluation of the samples showed that turbidity was present in all the tubes, being considerably less intense in the most concentrated ones (1/10 dilution), suggesting that these five solvents interfered with bacterial growth at high concentrations, but did not achieve a complete inhibition of it.

Interestingly, the absorbance rates indicate:

In **TBME** both strains had a similar response. At 1/1000 and 1/100 concentrations, absorbance rates were significantly higher than the positive control, whereas, at 1/10 concentration the absorbance rates turned lower than the control, indicating that at high concentration TBME were capable of restraining bacterial growth of the strains essayed.

In **CH_2Cl_2**, at 1/1000 concentrations absorbance rates were similar to the positive control. At 1/100 concentration, while in *Staphylococcus pseudintermedius* we find a much higher rate than the positive control, in *Pseudomonas aeruginosa* were just similar to it.

When the concentration increases, both rates decreased under the positive control, remarkably in *Staphylococcus pseudintermedius*, indicating that while bacterial growth of both strains have been compromised, *Pseudomonas aeruginosa* tolerated higher rates of this solvent. .

Analyzing **DMF** rates it showed that *Pseudomonas aeruginosa* tolerated much higher concentrations of it than *Staphylococcus pseudintermedius*. At 1:1000 concentration *Staphylococcus pseudintermedius* rates are very similar to the control, while in *Pseudomonas aeruginosa* were considerably higher.

When the concentration increased to 1:10, both strains showed a remarkably drop of the rates under the control, indicating a restriction of bacterial growth.

In case of **DMSO,** in both 1:1000 and 1:100 concentrations the absorbance rates were higher than the positive control in both strains. However, when the concentration increased to 1:10, *Staphylococcus pseudintermedius* experimented a remarkable drop of the values down the control, while *Pseudomonas aeruginosa* remained similar to the control, indicating that toxicity of DMSO in *Staphylococcus pseudintermedius* was more intense than in *Pseudomonas aeruginosa*, which showed a higher tolerance.

About **THF**, *Staphylococcus pseudintermedius* showed higher sensitivity to the solvent, being the absorbance rates similar to the control at 1:100 concentrations and much

lower at 1:10 concentration. On the other hand, *Pseudomonas aeruginosa* showed high tolerance, remaining similar to the positive control at 1:10 concentration.

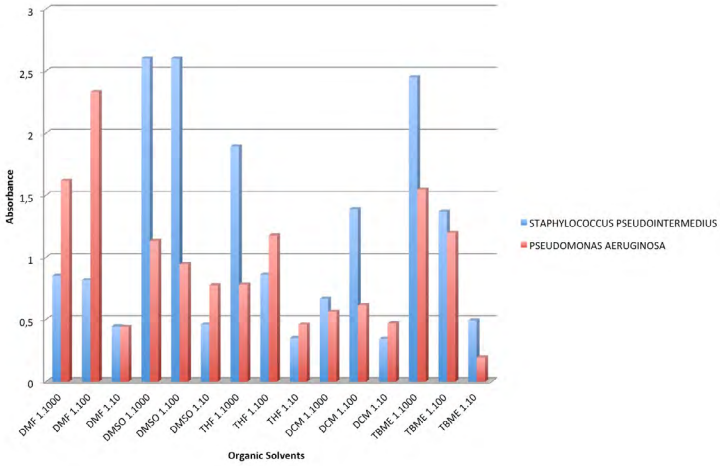

Figure 1. Results of absorbance.
Source: own elaboration.

4. Discussion and Conclusions

Analyzing the toxicity of the most frequent organic solvents used in chemical synthesis is indispensable to continue with further investigation of new molecules to inhibit bacterial growth.

The aim of this study is to establish the limits of concentration for bacterial toxicity of five organic solvents and analyze the behavior of bacterial growth in presence of foreign substances.

Analyzing the results we can conclude that the 1:1000 concentration is the one that is safe to use to solve hydrophobic substances, since it guaranteed good bacterial toleration. When not possible, the best option for *Staphylococcus pseudintermedius* is using DMSO or TBME at 1/100 dilution. By contrast, the best solvents for solving organic compounds for *Pseudomonas aeruginosa* were DMF and TBME at 1/100 dilution.

We consider the present study a first and very necessary step in the development of a new generation of substances to battle antibiotic resistant bacteria.

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