Simultaneous comparative study of several antimicrobials against several microorganisms

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Abstract

Antibiotic resistance represents one of the most significant threats to global health. To prevent ineffective prescriptions that exacerbate the selection of resistant strains, it is often necessary to use combinations of two or more antibiotics. However, these combinations may fail to achieve the desired clinical outcome if pharmacological antagonism occurs. Therefore, it is essential to characterize the nature of interactions within antibiotic mixtures in vitro before any therapeutic application.

Using a factorial design of experiments, we have developed a methodology to simultaneously study multiple antimicrobial substances and detect the nature of their interactive effects.

A polynomial model, $Y = f(X_1, X_2, \ldots X_n)$, links the effects of the antimicrobial substances (X_i) to the experimental response (Y), defined as the number of bacteria surviving the simultaneous action of these substances. This mathematical model provides significant insights:

Main Effects: The signs of the main effect coefficients X_i indicate which substances actively exert antimicrobial activity against the target bacteria.

Potency Ranking: Comparing the absolute values of these X_i coefficients allows for the classification of substances in order of increasing antimicrobial activity. This "screening" process ensures only the most effective agents are retained.

Interaction Dynamics: The (+) or (-) signs of the $X_i \cdot X_j$ interaction effects allow for the assessment of synergistic or antagonistic relationships. This enables researchers to prioritize effective combinations and rule out those that may be deleterious to the patient.

By utilizing a single experimental matrix, the resulting mixture can be tested against various bacterial strains, which can then be categorized by their sensitivity to each antibiotic molecule.

Initial objective

To develop a methodology, based on factorial experimental design, that allows for the simultaneous evaluation of the antibacterial activity of three antibiotics and the detection of synergistic or antagonistic interactions between them.

Material and methods

Factorial experimental design methodology is applicable in any field where a response variable **Y** depends on several independent variables ($\mathbf{x_i}$). The relationship can be expressed as [1] :

$$\mathbf{Y = f(x_1, x_2, \ldots x_n)}$$

In this study, the methodology was adapted such that :

The experimental response **Y** represents the growth level of the target microorganism.
The influencing factors $\mathbf{x_i}$ represent the antimicrobial substances.

A polynomial model is assigned to a specific bacterium, correlating the number of survivors to the quantities of the three antibiotics [2,3]:

$$\mathbf{Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{12}\,X_1 \cdot X_2 + a_{13}\,X_1 \cdot X_3 + a_{23}\,X_2 \cdot X_3 + a_{123}\,X_1 \cdot X_2 \cdot X_3 + \varepsilon}$$

Where :

Y : The number of surviving bacterial colonies after treatment with the three antibiotics.
X_1, X_2, and X_3 : The reduced or coded variables corresponding to each of the three antibiotics, respectively.
a_0 : The model constant.
a_1, a_2, and **a_3 :** The main effects or parameters that give the "weight" of antibiotics **X_1**, **X_2**, and **X_3**, respectively, on the bacteria.
a_{12}, a_{13}, and **a_{23} :** The synergistic or antagonistic interaction effects between two antibiotics.
a_{123} : The interaction effect between the three antibiotics.
ε : The experimental error term, considered as a random variable with zero mean and constant variance.

The model will be simplified after calculation by eliminating statistically insignificant coefficients.

Experimental domain

Each of the 3 factors (X_i) is assigned two levels (U_{inf} and U_{sup}, expressed in mg) which define its experimental range. All trials in a factorial experimental design are performed with U_{sup} values denoted (+1) and U_{inf} values denoted (-1).

Choice of factorial experimental design

To study the three antimicrobial substances (X_1, X_2, and X_3) simultaneously, a complete factorial experimental design of $\mathbf{2^3 = 8}$ trials was used to identify all possible interactions (Table 1).

Table 1: Factorial Experimental Design 2^3

Mixture N°	Antibiotic X_1	Antibiotic X_2	Antibiotic X_3	Response Y (Surviving Bacteria)
1	-1	-1	-1	Y_1
2	+1	-1	-1	Y_2
3	-1	+1	-1	Y_3
4	+1	+1	-1	Y_4
5	-1	-1	+1	Y_5
6	+1	-1	+1	Y_6
7	-1	+1	+1	Y_7
8	+1	+1	+1	Y_8

This matrix guides the preparation of antibiotic mixtures by weight. Each of the 8 mixtures is adjusted to a final volume of 10 ml using an appropriate solvent.

Culture technique

A direct contact method in liquid medium was chosen [2,3]. Eight test tubes, each containing 10 ml of liquid culture medium, received 1000 µl of one of the eight combinations of the three antimicrobials. The tubes were vortexed and inoculated with 100 µl of a 24-hour culture of the bacteria, then incubated at 37°C for 24 h.

After the 24-hour incubation period, one microliter is taken from each tube to perform a subculture on a Petri dish containing nutrient agar. The 8 subcultures are incubated at 37°C for 48 h. The antimicrobial activity is then estimated by counting the number of surviving bacteria **(y_i)**. The logarithm of these numbers constitutes the experimental responses **($Y_1, Y_2 \ldots Y_8$)**.

Estimation of model coefficients

The experimental responses **($Y_1, Y_2 \ldots Y_8$)**, specifically the logarithm of the number of bacterial colonies surviving the action of these antimicrobial combinations, allow for the calculation of the model coefficients by multiple regression in the least squares sense [1]

Interpretation of results

Analysis of the polynomial model allows us to :

► **Identify active agents:** Antibiotics that actually exerted an antimicrobial effect on the target bacteria are assigned a negative coefficient.

► **Rank efficacy:** Higher absolute values of the coefficients indicate more pronounced antimicrobial potency.

► **Determine interaction nature:** An interaction coefficient $\mathbf{X_{ij}}$ with a negative sign **(-)** reflects a **synergistic** effect between two antibiotics.

An interaction coefficient X_{ij} with a positive sign (+) reflects an **antagonistic** effect between two antibiotics.

► **Evaluate complex interactions:** If the coefficient $\mathbf{X_{ijk}}$ for the interaction between all three antibiotics is significant and negative, a synergistic interaction exists between the three.

Conclusion

The developed method allows for the in vitro testing of three combined antimicrobials on a bacterium.

Analyzing the coefficients of the mathematical model allows these antimicrobials to be ranked in order of increasing efficacy. This is crucial for therapeutic selection, as it ensures only the most effective agents are retained while eliminating those that could induce the selection of resistant mutant bacteria.

Furthermore, the method provides valuable information on the nature of synergistic or antagonistic interactions. This helps guide the choice of molecules to combine, avoiding the use of combinations that could be detrimental to the organism being treated. This modeling approach can be optimized and generalized to several different bacterial strains by using the same mixture prepared according to the matrix in Table 1, yielding specific mathematical models for each pathogen studied.

bibliographic references

[1] Box GEP, Hunter JS, Hunter WG (2005) Statistics for experimenters, Design, Innovation and Discovery, 2° Edition, John Wiley & Sons, New York. 664 p

[2] Belaiche T., Leriche F., (2021). Etude comparative simultanée de plusieurs antimicrobiens envers plusieurs microorganismes. *Industries Alimentaires et Agricoles*, Septembre/octobre, 44-46.

[3] Belaiche T., Leriche F., (2019). Détermination des effets synergiques ou antagonistes de plusieurs substances antimicrobiennes : application d'un plan d'expériences factoriel. *Phytothérapie*, Lavoisier 17, 235-239