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MICROBIAL INHIBITION TEST AS A MODERN METHOD OF IDENTIFICATION RESIDUAL ANTIBIOTICS (МІКРОБІОЛОГІЧНИЙ СКРИНІНГ ЯК СУЧАСНИЙ МЕТОД ВИЗНАЧЕННЯ ЗАЛИШКОВИХ КІЛЬКОСТЕЙ АНТИБІОТИКІВ)

У статті приділено увагу методам дослідження залишкової кількості антибіотиків, а саме мікробіологічному скринінгу. Даний тип скринінгу дозволяє оцінити рівень антибіотикорезистентності тест-культур мікроорганізмів, використовуючи порівняльний аналіз впливу різних концентрацій розчинів еталонних антибіотиків.

Ключові слова: *залишки антибіотиків, антибіотикорезистентність, тест-штами, мікроорганізми, індикатори.*

The article focuses on the methods of studying the residual amount of antibiotics, namely microbiological screening. This type of screening allows to estimate the level of antibiotic resistance of test cultures of microorganisms using a comparative analysis of the effect of different concentrations of solutions of reference antibiotics.

Keywords: *antibiotic residues, antibiotic resistance, test strains, microorganisms, indicators.*

Screening methods are used as more affordable and easy-to-use equipment to identify a group of antibiotics or an antibiotic, with or without quantification of this antibiotic. In general, analytical methods for monitoring antibiotic residues can be divided into two classes: confirmatory and screening. Confirmatory methods are used to quantify the concentration of an analyte based on liquid chromatography (LC) in combination with mass spectrometry.

Microbial Inhibition Test (MIT) is a method used to detect biologically active substances, in particular antibiotics, by assessing their effect on the growth of test cultures of microorganisms. This method is based on the incubation of medium plates with a suspension of a known concentration of bacterial strains, which is added to the sample under test. If there is an antibiotic in the sample, it will prevent the development of specific colonies, thus opening a halo zone around the sample to be analyzed. This type of screening is widely used to control antibiotic residues in food, feed, biological fluids and the environment, as their uncontrolled accumulation can lead to serious consequences, such as the development of antibiotic resistance, disruption of normal human microflora, allergic reactions and toxic effects [2, 3].

One of the important aspects of microbiological screening is the need for strict standardization of all test parameters, including incubation conditions, temperature, composition of culture media, and concentrations of standard antibiotic solutions, as even minor deviations can affect the accuracy of the results [1, 4].

A key advantage of microbiological screening compared to more sophisticated analytical methods such as liquid chromatography (namely, HPLC) or mass spectrometry is that it is more affordable and does not require expensive equipment, making it convenient for mass use. Sensitive bacterial strains are most often used for research, among which the most common are the genera Bacillus and Micrococcus. The main disadvantage of microbiological screening is the significant time spent on sample preparation and incubation of test Petri dishes [4].

Microbiological screening methods based on the principle of inhibiting the growth of microorganisms fall into two main categories: petri dish methods and test tube methods. Both approaches are aimed at detecting residual concentrations of antibiotics in the tested samples, but they have significant differences in their implementation, operating principles, sensitivity, and the ability to classify antibiotics [5].

Methods that use Petri dishes are based on the use of agarized nutrient media containing test

bacteria that are sensitive to antibiotics. The test samples, which may contain residual amounts of antimicrobial drugs, are placed on the surface of the agar medium. After incubation for 18-24 hours under favorable temperature conditions, a zone of bacterial growth inhibition can be observed around the sample if the concentration of antibiotics in it exceeds the established threshold. If the concentration of residual antibiotics is insufficient to inhibit the growth of microorganisms, there is no inhibition zone [3, 5].

An important feature of the Petri dish method is the ability not only to qualitatively determine the presence of antibiotics in the sample, but also to quantitatively analyze it. In addition, this method provides the ability to identify and classify antibiotics by using several Petri dishes with different pH values and specific bacterial strains. Additionally, special substances can be used to block or enhance the activity of certain groups of antibiotics, which allows them to be assigned to a specific class [1, 4].

Unlike methods that use Petri dishes, test tube methods (*Delvotest*) are based on the use of color indicators that respond to the presence or absence of antimicrobial substances in samples. Test bacteria and a corresponding indicator are added to the agar medium in the test tubes, which can change its color depending on the activity of the microorganisms. Typically, oxidation-reduction indicators or pH indicators are used, which allow for a visually noticeable result [4-5].

Although test tube methods are convenient and fast, they have limited sensitivity to some classes of antibiotics, in particular quinolones, which are widely used in veterinary medicine. In addition, the test tube method does not allow for the classification and identification of antibiotics, as it only indicates their overall presence in the sample [5].

Instead, the Petri dish method, although it takes longer to obtain results, has a significant advantage in accuracy, the ability to quantify residual levels of antibiotics and identify their classes. The use of special nutrient media, different pH conditions, and specific test cultures allows differentiating antibiotics and determining their belonging to a particular pharmacological group [2, 5].

Conclusions: Microbiological screening methods play a crucial role in detecting antibiotic residues in various samples, offering a cost-effective and accessible alternative to advanced analytical techniques such as liquid chromatography and mass spectrometry. The Petri dish method, based on bacterial growth inhibition, provides not only qualitative but also quantitative results, allowing for the classification of antibiotics. Despite its longer processing time, it ensures higher accuracy and specificity. On the other hand, test tube methods, while offering faster results, are limited in sensitivity and classification capability. Given the growing concerns about antibiotic resistance and the need for strict standardization, microbiological screening remains an essential tool in ensuring food safety, environmental monitoring, and public health protection.

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