Journal of Research in Medical and Dental Science
2020, Volume 8, Issue 7, Page No: 113-118

Available Online at: www.jrmds.in
eISSN No. 2347-2367: pISSN No. 2347-2545

Methodology for Determining Sensitivity of Microorganisms to Sorption Drugs

Vladimir Buhanov, Alexander Vezentzev, Spichak Irina, Ulyana Krut*, Tatyana Shevchenko, Larisa Kadutskaya, Elena Arseenko

Belgorod State National Research University, Belgorod, Russia

ABSTRACT

This study was undertaken to design methodological recommendations for determination of sensitivity of microorganisms to sorption drugs. The basic premise of it was long-term experiments that give optimal solutions for the studied issue. To our knowledge, no research has been carried out on the methodology for determination of sensitivity of microorganisms to sorption drugs. Realizing this gap, it is needed to create a modern clinically reasonable development based on the existing methodological instructive regulations 4.2.1890-04 "Determination of the sensitivity of microorganisms to antibacterial drugs". The study is focus on the most significant statements of determining the sensitivity of in vitro microorganisms to sorption drugs by the method of serial dilutions in a liquid nutrient medium. We have discussed the questions about interpretation of the results of determining the sensitivity of microorganisms to sorption drugs from a clinical and microbiological point of view. Our finding reveal that the developed methodological recommendations systematize the modern approaches of determination of the sensitivity of bacterial pathogens to sorption drugs, taking into account the recommendations of the European Committee for the Determination of Sensitivity to Antibiotics, as well as the US National Committee for Clinical Laboratory Standards.

Key words: Sorption preparations, Determination of the sensitivity of microorganisms, Serial dilution method

HOW TO CITE THIS ARTICLE: Vladimir Buhanov, Alexander Vezentzev, Spichak Irina, Ulyana Krut, Tatyana Shevchenko, Larisa Kadutskaya, Elena Arseenko, Methodology for Determining Sensitivity of Microorganisms to Sorption Drugs, J Res Med Dent Sci, 2020, 8 (7): 113-118.

Corresponding author: Ulyana Krut

e-mail✉: krut@bsu.edu.ru

Received: 01/10/2020

Accepted: 23/11/2020

INTRODUCTION

Strategic way for effective application of antimicrobial drugs in the infectious diseases is the determination of pathogen sensitivity for the drugs. It helps to choose the most resulting therapeutic agent of a great number of them turned out by biological industry and to perform replacement of the long-used drug by another if microbial resistance is registered for it. The determination of sensitivity is also carried out during monitoring the spread of resistance among microorganisms and in the process of studying new drugs [1].

Nowadays, in clinical practice two principle of antimicrobial drugs prescription exist: Empiric and etiotropic. Empiric prescription is based on the knowledge of the natural sensibility of bacteria, epidemiological data on the resistance of microorganisms in a region or a hospital, and the results that are controlling clinical research. Definitive advantage of the empiric prescription of the chemotherapeutic agents is possibility of the rapid onset. An additional point is that the approach eliminates the cost of additional studies. But in many cases of the ineffectiveness of the antibiotic therapy is carried out in the nosocomial infections when it is difficult to suggest the pathogen and its sensitivity to antibiotics etiotropic therapy is proceeded. Etiotropic prescription of antibiotics includes not only isolation of the pathogen from the clinical material, but also the determination of its sensitivity to antibiotics.

It is possible to obtain the correct data only with the competent performance of all links of the bacteriological study: sample collection, transportation of it into bacteriological laboratory, identification of the pathogen and determination its sensitivity to antibiotics and, finally, interpretation of the results [2].

Integrated approach for the assessment

of sensitivity and interpretation of the results designed by European Committee on Antimicrobial Susceptibility Testing (EUCAST) is well substantiated. EUCAST allows to use the traditional term “antibiotic” along with the most correct term “antimicrobial agent (drug).

EUCAST includes substances of natural, semisynthetic, or synthetic origin (The last one strictly refers to chemotherapeutic agents), exhibiting selective activity against bacteria, and potentially applicable for the treatment of infected people and animals into antibiotic group [3]. Antiseptics, disinfectants, and preservatives are not belonged into antibiotics.

Methods for determination sensitivity of bacteria to chemotherapeutic agents are divided into 2 groups: Diffusion and dilution methods. Diffusion methods includes that one with roundlet with antibiotic substances and with the help of E-tests. Sequential dilution assay is divided on dilution in the liquid medium (broth) and dilution in agar. These methods are based on using double stepwise dilution of antibiotic drug from maximal to minimal concentration. Basically, special mediums for determination of sensibility with pH 7.2-7.4 permitted for applying in Russian Federation are used. Assessment of bacteria sensibility with common nutritional needs is done by using Mueller-Hinton agar (MHA) or Mueller-Hinton broth (MHB) without extra supplements.

2-5% mechanically defibrinate horse or sheep blood and 20 mg/l β-NAD (nicothineamidedinucleotide) are added into agar or broth for fastidious organisms. The blood is lysed by freezing and defrosting procedure with the following centrifugation for liberation from achromacytes. Listed supplements are added in the medium after autoclaving and cooling till 48-50°C [4,5].

Any above-mentioned method can apply for determination of microorganism sensibility except but need to pay attention only on dilution ratio of weighted amount of the drug. However rapidly developed resistance of pathogens to antibacterial compounds, the immunosuppressive properties of drugs, microecological disturbances and the increasing etiological role of opportunistic pathogens under the influence of therapeutic agents’ motive researchers to search for new ways to optimize the treatment process [6,7].

Antibiotic resistance of pathogenic microorganism is a natural phenomenon of intensive and irrational use of antibacterial drugs [8]. Advanced direction for solving these problems is the using of natural sorbents that are safe for humans and animals. They inactivate pathogenic microorganisms and remove products of their vital activity, as well as products of impaired metabolism and toxic compounds obtained from the external environment.

As a result of the development of modern science, conventional medical technologies have been replaced by methods of efferent therapy (from the Latin "efferent"-"deduce"). They increase the effectiveness of traditional treatment, and sometimes completely replace it. These methods are available and are amazingly effective, since with the help of sorbents and complex preparations based on them, you can correct the state of the wound, the function of the gastrointestinal tract and reduce the overall toxic load on the body.

As a result of the development of modern science, received medical technologies have been replaced by methods of efferent therapy (from the Latin "efferent" - "deduce"). They increase the effectiveness of the traditional treatment and replace it completely sometimes. These methods are available and are amazingly effective because it is possible to corrective the state of the wound, the function of the gastrointestinal tract and reduce the overall toxic load on the body with the help of sorbents and complex preparations based on these methods [9,10].

In this case the sorbent does not react with sorbed substance and does not cause biochemical changes in the blood. The process of physiological filtration and reabsortion of the fluid from the blood stream into the intestinal lumen by its villi is used. Products that came out with the liquid part of the blood contact with the enterosorbent taken before. They are fixed on it and are excreted with it from the body.

Because intestinal villi can pass through all ingredients with molecular weight is lower that of albumin, and, basically, toxic substances have medium molecular weight the effectiveness of enterosorption in the elimination of endotoxemia

becomes clear [11].

A negative factor in several sorbents is the sorption of vitamins, mineral salts, and other useful substances, as well as non-specific sorption of enzymes (pepsin, trypsin, amylase), which requires correction of replacement therapy with enzyme drugs. It was identified that colloidal silicon dioxide in therapeutic doses does not cause noticeable changes in the activity of enzymes of the intestinal mucous membranes; differs in smaller (in comparison with other sorbents) excretion of vitamins and microelements and quick recovery of normal levels without additional drug load during studying long-term administration of the drug in different doses [12].

The competent and predicted use of sorbents is achieved by giving them selectivity by immobilizing specific ligands and receptors on their surface. A very wide range of compounds can be used as such ligands: Micro-and macro-elements, metal clusters, vitamins, enzymes, affinity receptors for specific biomolecules (e.g., cholesterol), adaptogens, dietary supplements, antibiotics, cytokines, immunomodulators, immunoglobulins, antigens, viruses and bacteria or their fragments, etc.

The ligands can be immobilized quite tightly so that they will not be washed away from the surface of the sorbent and will not enter the blood. Conversely, ligands can be immobilized loosely, so that they are easily desorbed from the surface of the sorbent and enter the body, for example during enterosorption [13].

Adsorbents are classified by sorption mechanism to absorbents with activated carbon, ion-exchange materials, sorbents with catabolic properties, sorbents with combined mechanisms. Others are divided by selectivity - selective, mono-, bi-, multifunctional, non-selective (charcoal, natural drugs - lignin, chitin, cellulose) [14].

Enterosorbents include drugs of various structures that are capable of binding substances, supramolecular complexes and cells by adhesion, adsorption, absorption, ion exchange and complexation. All enterosorbents can be divided into 4 main classes:

Coal (Various brands of charcoal).

Fibrous (Dietary fiber and their derivatives).

Mineral (Clays, zeolites, etc.).

Synthetic polymers (polyvinyl, organosilicon sorbents) [15]. It is necessary to create a modern clinically reasonable development based on the existing methodological instructive regulations 4.2.1890-04 “Determining the sensitivity of microorganisms to antibacterial drugs” due to the lack of a methodology for determining the sensitivity of microorganisms to the sorption drugs.

PURPOSE OF THE STUDY

We have designed the methodological recommendations for determination of the microorganisms’ sensitivity to sorption drugs. The motivation for this research was the following reasons:

In determining the sensitivity of microorganisms, the concentration of the sorbent is calculated not in μg/ml, but in mg/ml of the nutrient medium in contrast to the antibacterial drug.

In carrying out these studies on solid medium leads to increased cost and it requires larger amount of glassware (flasks, tubes, Petrie dishes).

It is painstaking job touched with serial dilution of sorbent in the melted agar, it is accompanied by rapid solidification of warmed agar and uneven suspension of the sorbent often.

The use of discs does not give trustworthy results due to the complication of the sorbent applying to the disk that is made of filter paper and the lack of diffusion of the sorbent in agar.

EXPERIMENTAL

Preparation of tubes with MHB and Petri dishes with MHA

MHB and MHA are prepared and autoclaved according manufacturer's instructions. Before autoclaving MHB was poured into flasks (400 ml), conical flasks (250 ml) and test tubes in volumes 5 and 9 ml. During the preparation MHA was poured into flasks (250 ml). After autoclaving the MHA was cooled to 42-45°C and poured into Petri dishes sterilely in such a way as to make agar thickness 4 ± 0.5 mm. The surface of the agar must be dry before use. If necessary, the agar was dried, the drying time may depend

on storage conditions and the environment. Do not over dry agar.

Sequential dilution assay

The sensitivity of the reference test cultures of opportunistic microorganisms to sorption-active drugs was determined in a liquid nutrient medium (MHB) by using the standardized sequential dilution assay.

Dilution of the studied sorption-active materials was carried out in the MHB.

Each sequence of dilutions is consisted of 8 tubes containing 5 ml of MHB. In the first tube the concentration of the sorbent was 200 mg/ml of MHB and in the subsequent one two times less.

Pre-prepared samples (weighing was on an analytical balance) of sorption-active materials were placed in tubes in the following quantity: in the first tube -1000.0; in the second - 500.0; in the third - 250.0; in the fourth - 125.0; in the fifth - 62.5; in the sixth - 31.25; in the seventh, 15.63; in the eighth - 7.82 mg. Then the tubes with weighed samples of sorbents were closed with silicone stoppers that can withstand temperatures up to 250°C and they were sterilised in the drying chamber at a temperature of 160°C for 20 minutes.

If a complex drug is made based on a natural sorbent, then the heat-labile drug is applied on the sorbent after its sterilization. Then 5 ml of sterile MHB and 0.1 ml of the studied daily bacterial culture (1 million microbial cells), which is 200000 microbial cells per 1 ml of MHB (2 x 105 CFU, i.e., colony forming units in 1 ml of MHB) were added to each tube.

Concentration of microorganisms was found by using a device for determining the turbidity of a bacterial suspension Densi-La-Meter II, the principle of which is based on the optical absorption of the suspension with the issuance of the measurement result in units of MacFarland.

Inoculum preparation

The inoculum was prepared using the direct suspension method of a pure 18-24-hour culture of bacteria colonies grown on a dense non-selective nutrient medium in a sterile isotonic sodium chloride solution. The bacterial suspension was inoculated in tubes with dilutions of the sorbent for 15 minutes but no later than 60 minutes after preparation.

The concentration of the sorbent in the MHB from the first to the eighth tube of each sequence, respectively, was: 200.0; 100.0; 50.0; 25.0; 12.5; 6.25, 3.13 and 1.56 mg/ml. The 9th and 10th tubes were used as a control. The 9th tube contained only 5 ml of MHB and the 10th - 5 ml of MHB and 1 million microbial cells of the studied reference test culture.

The reference bacterial strain was not added in the second control series of identical dilutions ("negative" control) of sorption-active materials in 5 ml of MHB (8 tubes).

Incubation

Properly prepared contents of test and control tubes were suspended with a stirrer - Vortex V-1 plus. Then it was cultured for 16-18 hours in a thermostat at a temperature 37 °C. Then, the obtained findings were considered.

Control the results of determining the sensitivity of microorganisms to sorbents.

The minimum concentration of sorbent (mg/ml) which suppressed the visible growth of microorganisms completely is taken as minimal inhibitory concentration (MIC). The established MIC of the sorbent is considered bacteriostatic. The reliability of the result is confirmed by the testimony of the Densilameter, which determines the optical density of the suspension in identical dilutions of the sorbent of the experimental and control series of tubes.

In some cases, when the high concentration of sorption-active material in the MHB (from the first to the third test tube of the experimental and control series) does not allow using the densimeter due to the large precipitation of sorbent formed at the bottom of the tube. The super sedimentation density of the suspension in the MHB is determined by visual comparison with the standard McFarland turbidity on a white background with black lines. The McFarland turbidity standards are a collection of test tubes with an increasing concentration of barium sulfate. The turbidity of the suspension formed by the white barium sulfate precipitate is a value corresponding to a specific concentration of the bacterial suspension. The set contains 5 tubes (one tube of each McFarland standard: 0.5, 1, 2, 3, and 4 units). Before using the turbidity, standard should be vigorously shaken on the vortex. (When using commercial turbidity

standards, it is necessary to carefully study the manufacturer's instructions: some of them should not be shaken, since they have a gel base). The difference between the densitometer and the McFarland turbidity standards of the experimental and control tubes with two dilutions of sorbents is judged the ability of the studied samples to inhibit the microorganism growth.

The turbidity of the bacterial suspension measured in McFarland units, will correspond to the CFU of viable microorganisms per unit volume (1 cm3), in liquid (1 ml), in a particular case in 1 ml of MHB approximately. The research has been done within the framework of State Assignment of the Russian Federation Ministry of Science and Higher Education № FZWG-2020-0021.

RESULTS AND DISCUSSION

Establishing minimum bactericide concentration (MBC) in studied sorbents

According to the aim to identify the bactericidal effect of the studied sorbents 0.1 ml of suspension was taken from the contents of the last three tubes of the test series, where there was no visible growth of the test culture with a sterile pipette, and cultures were inoculated on MHB or meat infusion broth. After that they were cultivated for 16-18 hours in an incubator at temperature 37 °C. The lowest concentration of sorbent in the test tube where the inoculum was inoculated shows MBC if there was no formation of colonies on agar or in broth.

The determination of the sensitivity of microorganisms for the studied sorbents was carried out 3 times, until comparable results were obtained. The 10th tube with the presence of the growth of microorganisms was served as a control of the growth of the test culture in each experimental sequence, and the 9th tube of the series was served as the MHB sterility control. An intact series of tubes (8 tubes without inoculum) was the control of sterile 2-fold dilutions of the sorbent in the MHB.

CONCLUSION

Thus, the developed methodological recommendations systematize modern approaches for determination the sensitivity of bacterial pathogens of infectious diseases of humans and animals to sorption drugs, taking into account the recommendations of the European Committee for the Determination of Sensitivity to Antibiotics, as well as the National Committee for Clinical Laboratory Standards of the United States.

ACKNOWLEDGMENT

The study was done in the certified laboratory of the National Research University "BelSU" for inflectional safety.

REFERENCES

1. Volkova PM, Antonov BI, Hrapova VE, et al. Laboratory studies in veterinary medicine. Moskva: Kolos 1971.
2. Reshedko GK. Determination of sensitivity to antibiotics: methods, results, assessment. Clin Antimicrob Chemotherapy 1999; 3:113-115.
3. Subcommittee on antifungal susceptibility testing (AFST) of the ESCMID European committee for antimicrobial susceptibility testing (EUCAST) Arendrup MC, Cuenca-Estrella M. Document E.DEF 7.2: Method for the determination of broth dilution of antifungal agents for fermentative yeasts revised March 2012.
4. Clinical recommendations “Determining the sensitivity of microorganisms to antimicrobial agents”/Approved: Meeting of the working group on medical microbiology of the relevant commission of the Ministry of Health of the Russian Federation on KLD (All-Russian Scientific and Practical Conference on Medical Microbiology and Clinical Mycology (XVII Kashkinsky Readings), 2014; 9-11, 2014.
5. http://docs.cntd.ru/document/1200038583
6. Ljubarskij MS. Combined lymphotropic and sorption therapy of purulent wounds. Bishkek–Novosibirsk 1995.
7. Ju L, Semchenko VL, Polujektov SS, et al. The rationale for the local use of sorption-detoxification therapy in postoperative purulent-septic complications in patients with colorectal cancer. Bjulleten 2002; 2:101-104.
8. Kozlov RS. Antimicrobials and resistance of microorganisms: two sides of the coin. Vedomosti Nauchnogo centra Jekspertizy Sredstv Medicinskogo Primenenija. 2007; 3:30-32.
9. Borodin I. Sorption-application and lymphotropic methods in the complex treatment of burns. Novosibirsk: 1995.
10. Lopatkin NA. Effective methods in medicine. Medicina 1989.
11. Nikolaev VG, Mihalovskij SV, Nikolaeva VV, et al. Enterosorption: State of the issue and prospects for the future. Bulletin Problems Biol Med 2007; 4:7-17.
12. Chuiko AA, Pogorelyj VK, Pentjuk AA, et al. Medical

chemistry and clinical use of silicon dioxide. Kiev 2003.

13. Burmistrov VA, Rachkovskaja LN, Ljubarskij MS, et al. Specific sorbents for the prevention and treatment of various diseases. 2007.
14. Belyakov NA, Solomennikov AV. Enterosorption (introduction to the problem) 1990.
15. https://on-line-wellness.com/