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Analysis of antibiotic sensitivity of clinical strains of microorganisms with the Russian Mueller–Hinton broth

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Abstract

Introduction. One of the reasons for spreading antibiotic-resistant microorganisms is the uncontrolled use and inadequate empirical prescription of antibiotics which is not based on the results of the pathogen sensitivity testing. The broth dilution method and one of its implementation options, the reference microdilution method, in contrast to the disk diffusion method, allows testing virtually all pathogen-antibiotic combinations. To realize the method, a production technology of Russian Mueller–Hinton broth (MHB-Obolensk) has been developed under the import substitution program.

The aim. To evaluate the quality of the developed domestic Mueller–Hinton broth in comparative tests with its imported analog BD BBL (MHB-BD) in testing clinical strains of microorganisms, including microorganism–antibiotic combinations pairs which cannot be reliably investigated by the disc diffusion method.

Materials and methods. The study investigated the sensitivity of 47 clinical strains of Gram-positive and Gram-negative bacteria to antibiotics of various functional groups using the broth microdilution method with MHB-Obolensk and MHB-BD.

Results. The MICs values of antibiotics for clinical strains obtained with the developed and control media did not practically differ from each other or differed by +/– one dilution. The difference by two two-fold dilutions was noted when testing *Enterococcus faecium*–ampicillin, *Klebsiella pneumoniae*–meropenem, *Pseudomonas aeruginosa*–levofloxacin and *Staphylococcus aureus*–ciprofloxacin combinations. For the first two combinations, the MIC values were lower in MHB-Obolensk, and for the last two, they were higher than in MHB-BD. The differences obtained did not affect the clinical categories of sensitivity.

Conclusion. The antibiograms of clinical strains in developed Russian Mueller–Hinton broth was obtained, which did not differ from those for the comparison medium. MHB-Obolensk complies with the requirements of national and international standards and can be used to reliably test, among other things, current combinations of microorganism–antibiotic pairs that cannot be studied using the disk diffusion method.

Keywords: *Mueller–Hinton broth, import substitution program, broth microdilution method.*

Ethics approval. Only museum strains of microorganisms were used in the study; therefore, no biomedical ethics committee opinion or other documents are required to be submitted.

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Оригинальное исследование
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Использование отечественного бульона Мюллера–Хинтон для исследования антибиотикочувствительности клинических штаммов микроорганизмов

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Аннотация

Введение. Одна из причин распространения микроорганизмов, устойчивых к антимикробным препаратам (АМП), связана с бесконтрольным употреблением и неадекватным эмпирическим назначением антибиотиков, не основанным на результатах определения чувствительности возбудителя к ним. Метод разведений в бульоне и один из вариантов его исполнения — референтный метод микроразведений, в отличие от диско-диффузионного метода, позволяет тестировать практически все комбинации патоген–антибиотик. Для выполнения метода в рамках программы импортозамещения разработана технология производства отечественного бульона Мюллера–Хинтон (МХБ-Оболенск).

Цель исследования — оценить качество разработанного отечественного бульона МХБ-Оболенск в сравнительных испытаниях с импортным аналогом МХБ-BD («BD BBL») при тестировании клинических штаммов микроорганизмов, включая комбинации микроорганизм–АМП, которые нельзя достоверно исследовать диско-диффузионным методом.

Материалы и методы. В работе исследовали чувствительность 47 клинических штаммов грамположительных и грамотрицательных бактерий к АМП различных функциональных групп методом микроразведений в бульонах МХБ-Оболенск и МХБ-BD.

Результаты. Значения минимальных подавляющих концентраций (МПК) антибиотиков для клинических штаммов, полученные на разработанной и контрольной средах, между собой практически не отличались или отличались на +/– 1 разведение. Отличие на 2 двукратных разведения отмечено при тестировании комбинаций *Enterococcus faecium*–ампициллин, *Klebsiella pneumoniae*–меропенем, *Pseudomonas aeruginosa*–левофлоксацин и *Staphylococcus aureus*–ципрофлоксацин. Для двух первых комбинаций значения МПК на МХБ-Оболенск были ниже, а для двух последних — выше, чем на МХБ-BD. Полученные различия не отразились на клинических категориях чувствительности.

Заключение. На разработанном отечественном бульоне МХБ-Оболенск получены антибиотикограммы для клинических штаммов микроорганизмов, которые не отличались от их антибиотикограмм на контрольной среде. МХБ-Оболенск соответствует требованиям национальных и международных стандартов и с помощью него можно достоверно тестировать в том числе актуальные комбинации пар микроорганизм–АМП, которые нельзя исследовать диско-диффузионным методом.

Ключевые слова: *бульон Мюллера–Хинтон, импортозамещение, метод микроразведений в бульоне*

Этическое утверждение. В исследовании использованы только музейные штаммы микроорганизмов, поэтому не требуется представления заключения комитета по биомедицинской этике или иных документов.

Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Introduction

The large-scale spread of bacteria resistant to various groups of antibiotics continues to be a public health problem worldwide [1]. The largest number of resistance cases is among healthcare-associated infections, including *Acinetobacter baumannii,* members of the *Enterobacterales* family*, Enterococcus faecium, Staphylococcus aureus*, *Pseudomonas aeruginosa*, etc. [2]. According to the estimates of experts, in 2019 alone, about 5 million cases of deaths caused by antibiotic-resistant bacteria have been identified worldwide [3], including multidrug-resistant or rifampicin-resistant tuberculosis[1].

The COVID-19 pandemic has exacerbated the existing global burden of antibiotic resistance mainly due to inappropriate and overuse of antibiotics [5].

The situation of the growing threat of antibiotic resistance is complicated by the significant decline in the development of new antimicrobials due to the lengthy procedure from development to implementation, high cost and low cost recovery. Currently, it takes about 10–15 years to advance a candidate antibiotic from preclinical to clinical trial stage [6]. Considering the critical necessity for new antibiotics, in 2017, the World Health Organization (WHO) published a list of resistant bacteria posing the greatest risk to human life and health[2], an updated list was released in 2024[3]. The updated list excludes 5 pathogen-antibiotic combinations (clarithromycin-resistant *Helicobacter pylori*; fluoroquinolone-resistant *Campylobacter* spp.; penicillin-resistant *Streptococcus pneumoniae*; third-generation cephalosporin-resistant *Providencia* spp.; vancomycin-resistant *S. aureus*) that were contained in the 2017 list, and added 4 new bacteria-antibiotic combinations: macrolide-resistant *Streptococcus group* A; penicillin-resistant *Streptococcus group* B; macrolide-resistant *S. pneumoniae*; rifampicin-resistant *Mycobacterium tuberculosis*. Carbapenem-resistant *P. aeruginosa* moved from the critical priority level to the high priority level due to reports of a decline in its global resistance to antibacterial drugs.

Another reason for the emergence of antimicrobial-resistant microorganisms is associated with uncontrolled and unjustified use of antibiotics, as well as inadequate empirical prescription of antibiotics without taking into account the results of sensitivity testing. Currently, the most common method of determining the sensitivity of microorganisms remains the disk-diffusion method. It is easy to perform and does not require expensive equipment. However, certain microorganism-antimicrobial combinations cannot be reliably tested by this method, which may lead to incorrect prescription of treatment regimens and further aggravate the situation with the spread of antibiotic resistance.

The broth dilution method and especially one of its variants, the microdilution method, which is recognized as a reference method, are free of such limitations[4]. This is a quantitative method, the use of which allows to determine the values of minimum inhibitory concentrations (MIC) of antimicrobials, which most accurately reflect the antimicrobial effect *in vitro* and are necessary to optimize the dosing regimen of antimicrobials.

The method allows testing such microorganism–antibiotic combinations, which cannot be reliably tested by disk-diffusion method, and some of which are included in the WHO list: *Salmonella* spp., resistant to ciprofloxacin; *Neisseria gonorrhoeae* resistant to cephalosporins and fluoroquinolones; *S. pneumoniae* and group A streptococci resistant to macrolides (azithromycin, clarithromycin and roxithromycin in case of resistance to erythromycin); non-brutonotyphoidal salmonellae resistant to fluoroquinolones (ciprofloxacin); etc.

It is recommended to use Mueller–Hinton broth (MHB) standardized for the content of divalent metal ions, thymidine and pH value because of their influence on the activity of some antibiotics. Until recently there was no industrial production of MHB in Russia, and the current situation with the imposition of economic sanctions against our country has led to the restriction of export of products for microbiological research. In this connection in the State Scientific Center of Applied Microbiology and Biotechnology, the production technology was developed and industrial production of MHB was established (RU RZN No. 2023/21584 from 29.11.2023). The broth has been tested on an expanded set of test strains and antimicrobials, and this study is devoted to studying the possibility of its use in testing clinical strains of microorganisms.

The aim of the study is to evaluate the quality of the developed domestic MHB in comparative tests with its imported analog BD BBL (MHB-BD) in testing clinical strains of microorganisms, including microorganism–antibiotic combinations pairs which cannot be reliably investigated by the disc diffusion method.

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[1] Tuberculosis: Multidrug-resistant (MDR-TB) or rifampicin-resistant TB (RR-TB). 2024. URL: https://www.who.int/news-room/questions-and-answers/item/tuberculosis-multidrug-resistant-tuberculosis-(mdr-tb)

[2] WHO publishes list of bacteria for which new antibiotics are urgently needed. 2017. URL: https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed

[3] WHO bacterial priority pathogens list, 2024: Bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance. 2024. URL: https://www.who.int/publications/i/item/9789240093461

[4] GOST R ISO 20776-1. Infectious agent susceptibility testing and evaluation of the functional performance of articles for antimicrobial susceptibility testing. Part 1. Reference broth microdilution method for laboratory testing of the activity of antimicrobial agents against fast-growing aerobic bacteria causing infectious diseases. Moscow; 2022. 24 p.

Materials and methods

Nutrient media

MHB produced by the State Research Center for Applied Microbiology and Biotechnology (MHB-Obolensk; cat. no. O-282-K-1) and MHB produced by BD BBL (MHB-BD; cat. no. 212322) were used in the study, the latter was used as a control medium. For testing fastidious microorganisms, 5% lysed horse blood and 20 mg/L β-NAD (Sigma-Aldrich, cat. no. N8535) were added to the broths. Lysed horse blood was prepared from defibrinated horse blood (Ecolab) by adding sterile deionized water in a 1:1 ratio to defibrinated horse blood, placing it in a freezer for 8 ± 1 h at –20ºC. The thawed blood was then re-frozen and thawed once more at room temperature, repeating this cycle 4 times until complete lysis of blood cells. The lysed horse blood was then clarified by centrifugation at 7000 r/s for 30 min on an Eppendorf Centrifuge 5702 machine.

Microorganism strains under study

The strains of microorganisms deposited in the State Collection of Pathogenic Microorganisms (SCPM-Obolensk) were tested:

- 44 clinical strains of microorganisms previously isolated from patients treated at the inpatient department of the Regional Infectious Diseases Clinical Hospital of Yaroslavl Region and deposited in the SCPM-Obolensk: 14 strains of *K. pneumoniae*, 8 strains of *P. aeruginosa*, 4 strains of *A. baumannii*, 7 strains of *Staphylococcus* spp. (*S. aureus* — 6, *S. epidermidis* — 1), 7 strains of *Enterococcus* spp. (*E. faecium* — 4, *E . epidermidis* — 1), 7 strains of *Enterococcus* spp. (*E. faecium* — 4, *E. faecalis* — 1*, E. casseliflavus* — 1, *E. gallinarum* — 1), 2 strains of *Escherichia coli*, 1 strain of *Corynebacterium pseudodiphtheriticum*, 1 strain of *Morganella morganii*;
- 3 Campylobacter strains isolated from bird droppings of a farm in Moscow region and deposited in the State Research Center for Applied Biotechnology and Microbiology, Obolensk (*C. jejuni* — 2, *C. coli* — 1);
- 5 test strains used for routine quality control of testing and broths investigated in the work: *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, *E. faecalis* ATCC 29212 and *C. jejuni* ATCC 33560.

Antimicrobials

The following drugs were used in the study: amikacin (cat. no. A1774), ampicillin (cat. no. A9393), vancomycin (cat. no. 94747), gentamicin (cat. no. G3632), imipenem (cat. no. I0160), colistin (cat. no. C4461), levofloxacin (cat. no. 28266), linezolid (cat. no. PHR1885), meropenem (cat. no. PHR1772), tetracycline (cat. no. T8032), tigecycline (cat. no. PZ0021), trimethoprim (cat. no. T7883), ceftazidime (cat. no. PHR1847), ciprofloxacin (cat. no. 17850), erythromycin (cat. no. E6376), sulfamethoxazole (cat. no. S7507) — all manufactured by Sigma-Aldrich.

Broth microdilution method

The method was performed using a 96-well plate in accordance with the requirements of GOST R ISO 20776-1[4], as well as current versions of EUCAST and Russian recommendations for determining the sensitivity of microorganisms to antimicrobials[5, 6]. The obtained MIC values were used to determine the sensitivity categories of strains: S (sensitive under standard dosing regimen), R (resistant), I (sensitive under increased antimicrobial exposure). Testing of all microorganism-antimicrobial combinations was performed in 3 repetitions.

Physicochemical indicators of nutrient media quality

Physicochemical parameters of broth quality (amine nitrogen content, chloride content in terms of NaCl and loss in weight during drying) were determined in accordance with Methodological Guidelines 4.2.2316-08[7]. The content of calcium (Ca^{2+}), magnesium (Mg^{2+}), manganese (Mn^{2+}) and zinc (Zn^{2+}) ions was determined by atomic emission spectrometry with inductively coupled plasma on the iCAP-6500 Duo plasma spectrometer (Thermo Scientific) in accordance with the requirements of GOST R ISO 27085-2012[8].

Thymidine content was evaluated by indirect method by determining the MIC value of trimethoprim/sulfamethoxazole in the study of control strain *E. faecalis* ATCC 29212. Obtaining an MIC ≤ 0.5/9.5 mg/L indicated an acceptable thymidine concentration of less than 0.03 mg/L in the broth[9].

Statistical methods

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[5] European Committee for Antimicrobial Susceptibility Testing (EUCAST). URL: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/QC/v_14.0_EUCAST_QC_tables_routine_and_extended_QC.pdf

[6] Russian recommendations "Determination of susceptibility of microorganisms to antimicrobial agents" (version 2024-02). KMAX. 2024;26(2). URL: https://microbius.ru/library/rossiyskie-rekomendatsii-opredelenie-chuvstvitelnosti-mikroorganizmov-k-antimikrobnym-preparatam

[7] Methodological guidelines 4.2.2316-08. 4.2. Methods of control of bacteriological nutrient media: Methodological guidelines. Moscow; 2008.

[8] GOST R ISO 27085-2012. Animal feeds. Determination of calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum, arsenic, lead and cadmium by ICP-AES method. Moscow; 2014.

[9] GOST P 59786-2021/ISO/TS 16782:2016. Clinical laboratory tests. Acceptability criteria for lots of dehydrated agar and Mueller-Hinton broth used for antibiotic sensitivity assessment. Moscow; 2021. 30 p.

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The results were processed using the MS Excel program package. The reliability of different mean values was assessed using Student's t-criterion. Two-tailed Fisher's exact test was used in comparative analysis, significance level $p < 0.05$.

For test strains of microorganisms, the obtained values of MIC of antibiotics were compared with target values and acceptable ranges. The obtained results in accordance with GOST R ISO 20776-2-2010[10] were presented in the following evaluation categories:

- C — the mean value corresponds to the target value;
- H — high, the mean value is higher than the target value by 1 twofold dilution;
- L — low, the mean value is lower than the target value by 1 twofold dilution;
- VH — very high, the mean value is higher than the target value by 2 twofold dilutions, but is within the range of acceptable values;
- VL — very low, the mean value is lower than the target value by 2 twofold dilutions, but is within the range of acceptable values;
- LE — low error, the mean value is less than the lowest acceptable value;
- HE — high error, the mean value is more than the highest acceptable value.

Results

Before the beginning of the study, the quality control of MHB-Obolensk was carried out using control strains of *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, *E. faecalis* ATCC 29212, *C. jejuni* ATCC 33560 and antibiotics, the results of sensitivity to which depend on the quality of the used MHB [9-11]. When selecting antibiotics, we proceeded from the following requirements of the standards: to obtain reliable results of sensitivity testing to tetracyclines, penicillins, aminoglycosides, macrolides and fluoroquinolones, it is recommended to use MHB with an optimal pH value of 7.2–7.4. For aminoglycosides, tetracyclines and fluoroquinolones the medium should be strictly balanced in terms of calcium and magnesium ions, for tigecycline and carbapenems – in terms of concentration of manganese and zinc ions, respectively, and for sulfonamide drugs the concentration of thymidine in the broth is critical.

During the quality control of MHB-Obolensk, the MIC values of the antibiotics obtained were classified as category C in 84.4% of cases. In 8.0% of cases the obtained MIC values of antibiotics were classified as category H, and in the remaining 7.6% of cases the obtained MIC values were qualified as L. No values categorized as VH, VL, LE and HE were obtained during the study. The MIC values of antibiotics for the test strains determined on the control medium MHB-BD were also within the permissible intervals. The results obtained testified to the high quality of the analyzed nutrient media and the possibility of their use for the study of clinical strains.

Upon further research on the developed and control broths, we studied the sensitivity of *Enterobacterales* representatives (*K. pneumoniae*, *E. coli* and *M. morganii*) to imipenem, meropenem, ceftazidime, levofloxacin, ciprofloxacin, ampicillin, colistin, gentamicin and trimethoprim/sulfamethoxazole, and *E. coli* — additionally to tigecycline, *P. aeruginosa* — to imipenem, meropenem, ceftazidime, levofloxacin, ciprofloxacin and colistin, *A. baumannii* — to imipenem, meropenem, levofloxacin, ciprofloxacin, colistin and gentamicin, *Campylobacter* spp. — to ciprofloxacin, tetracycline and erythromycin, *Staphylococcus* spp. — to levofloxacin, ciprofloxacin, linezolid, vancomycin, tetracycline, gentamicin, erythromycin, tigecycline and trimethoprim/sulfamethoxazole, *Enterococcus* spp. — to imipenem, levofloxacin, ciprofloxacin, linezolid, vancomycin, ampicillin and tigecycline, *C. pseudodiphtheriticum* — to ciprofloxacin, linezolid, vancomycin and tetracycline.

The MIC values of antibiotics obtained on MHB-Obolensk and control MHB-BD were almost identical to each other. When 8 antimicrobial-microorganism combinations were tested, differences in MIC per 1 two-fold dilution were noted. For 4 combinations on MHB-BD they exceeded the values on control broth and amounted to 0.12 mg/L vs. 0.06 mg/L for meropenem against *K. pneumoniae* 16, 0.06 mg/L vs. 0.03 mg/L for imipenem against *K. pneumoniae* 203, 0.25 mg/L versus 0.125 mg/L for ceftazidime against *E. coli* 1169/70, and 0.06 mg/L versus 0.03 mg/L for levofloxacin against *A. baumannii* 494. In contrast, they were lower for 4 combinations and were 0.03 mg/L versus 0.06 mL/L for levofloxacin on control broth against *E. faecalis* 2211406, 16.0 mg/L vs. 32 mg/L for tetracycline against *C. jejuni* F-2, 0.25 mg/L vs. 0.5 mg/L for colistin against *K. pneumoniae* 1643, 0.03 mg/L vs. 0.06 mg/L for vancomycin against *S. aureus* 2202263.

Differences between the two MHBs in MIC results at 2 two-fold dilutions were observed when 4 combinations were tested: *K. pneumoniae* 1142–meropenem, *P. aeruginosa* 265–levofloxacin, *S. aureus* 2202309–ciprofloxacin and *E. faecium* 613–ampicillin. At the same time, the MIC values of levofloxacin and ciprofloxacin, both equal to 0.12 mg/L, respectively, were higher on MHB-Obolensk than on control broth (0.03 and 0.03 mg/L), and the MIC values of meropenem and ampicillin, equal to 0.06 and 0.03 mg/L, respectively, were lower than on MHB-BD (0.016 and 0.008 mg/L).

However, the resulting differences in MIC val-

[10] GOST R ISO 20776-2-2010. Clinical laboratory tests and in vitro diagnostic test systems. Infectious agent susceptibility testing and evaluation of functional performance of products for antimicrobial susceptibility testing. Part 2. Evaluation of the functional performance of antimicrobial susceptibility testing products.

ues did not affect the evaluation of clinical sensitivity categories of the clinical strains tested. The results of antimicrobial sensitivity testing (in clinical sensitivity categories) for 44 clinical strains of microorganisms and 3 Campylobacter strains isolated from farm birds are presented in the **Table**.

All strains of *K. pneumoniae* studied in this work are mainly sensitive to the tested antibiotics under standard dosing regimen. One strain was resistant to ceftazidime and ciprofloxacin, 2 — to ampicillin, 3 — to gentamicin. Both *E. coli* strains were sensitive under standard dosing regimen to imipenem, meropenem, ceftazidime, levofloxacin, ampicillin, tigecycline and trimethoprim/sulfamethoxazole. One of them showed resistance to ciprofloxacin, colistin and gentamicin, while the other was sensitive to these antimicrobials. The *M. morganii* strain was interpreted as sensitive under standard dosing regimen to imipenem, meropenem, ceftazidime, levofloxacin, ciprofloxacin, ampicillin, colistin and trimethoprim/sulfamethoxazole, but resistant to gentamicin.

Antibiogram analysis of *P. aeruginosa* strains showed that all were sensitive to meropenem, and at increased antimicrobial exposure were also sensitive to imipenem, ceftazidime, levofloxacin, and ciprofloxacin. One of the 8 pseudomonad strains tested showed resistance to colistin, while the other 7 were sensitive to it.

A. baumannii strains were sensitive to imipenem, meropenem, levofloxacin, and colistin, and to ciprofloxacin at increased antimicrobial exposure. Only 1 strain was sensitive to gentamicin, and the others showed resistance, as did all 4 strains tested to trimethoprim/sulfamethoxazole.

One strain of Campylobacter showed resistance to ciprofloxacin, tetracycline and erythromycin, two others showed sensitivity to tetracycline and erythromycin and sensitivity, but with increased exposure, to ciprofloxacin.

All investigated gram-positive strains of *Staphylococcus* spp. and *Enterococcus* spp. are sensitive to linezolid. Against levofloxacin and ciprofloxacin, all *Staphylococcus* spp. strains were interpreted as sensitive at increased antimicrobial exposure, and against vancomycin and tigecycline as sensitive at standard dosing regimen. One of the 7 *Staphylococcus* spp. strains tested showed resistance to tetracycline and erythromycin, while the rest were sensitive under the standard dosing regimen. Regarding gentamicin and trimethoprim/sulfamethoxazole, only 4 strains of *Staphylococcus* spp. were sensitive to these antimicrobials, while the remaining 3 were resistant to them.

All *Enterococcus* spp. strains were sensitive to levofloxacin, ciprofloxacin and ampicillin at standard dosing regimen, and to imipenem — at increased exposure. At the same time, 3 strains of *E. faecium* showed resistance to vancomycin and tigecycline, while the other 4 strains showed sensitivity.

The *C. pseudodiphtheriticum* strain tested in combination with linezolid, vancomycin and tetracycline was interpreted as sensitive and in combination with ciprofloxacin as resistant. Using the control nutrient medium MHB-BD, similar antibiograms were obtained for all tested microorganism strains.

Discussion

In this study, the sensitivity of Gram-negative and Gram-positive bacteria (including fastidious bacteria) isolated from sick people and farm animals to antibiotics of different groups was tested on MHB-Obolensk. The list of antimicrobials included antibiotics, sensitivity to which cannot be determined by disk-diffusion method (ciprofloxacin only for salmonella, vancomycin and colistin for all microorganisms); antibiotics that act as quality markers for MHB (tetracycline, gentamicin, erythromycin, tigecycline, trimethoprim/sulfamethoxazole, levofloxacin, imipenem, meropenem, ampicillin) and others (linezolid, ceftazidime) [9–11].

At the State Research Center for Applied Microbiology and Biotechnology, we managed to design an MHB that meets the requirements of national and international standards (see footnotes 5, 8), based on a specially developed hydrochloric acid hydrolysate of modified casein. The pH value of the developed broth is in the range of 7.2–7.3, calcium ion content is in the range of 20.0–25.0 mg/L, magnesium — 10.0–12.0 mg/L, manganese level in it is less than 8.0 mg/L, zinc — less than 3.0 mg/L, thymidine — less than 0.03 mg/L. Other physicochemical quality parameters, the requirements for which are not regulated by the standard (see footnote 8), do not differ from those for the imported analog: the content of amine nitrogen varies from 4.7 to 5.0%, chlorides — from 27.5 to 28.7%, and the loss in weight during drying is 3.8–4.0%.

The use of MHB with such characteristics allowed to obtain the results of sensitivity categories of 47 clinical strains of microorganisms to 14 antibiotics, not differing from those on the control medium of a reliable manufacturer — MHB-BD.

The produced broth can be used for routine performance of serial dilutions method in macro- and micro versions, for commercial tests (in the format of tablets and MIC-strips with dried antibiotic substances), as well as for automatic analyzers.

Conclusion

The antibiograms of clinical strains in developed Russian Mueller–Hinton broth was obtained, which did not differ from those for the comparison medium. MHB-Obolensk complies with the requirements of national and international standards and can be used to reliably test, among other things, current combinations of microorganism–antibiotic pairs that cannot be studied using the disk diffusion method.

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Results of clinical strain testing by broth microdilution method using MHB-Obolensk and MHB-BD

Antibiotics	Nutrient media	*K. pneumoniae* — 14		*E. coli* — 2		*M. morganii* — 1		*P. aeruginosa* — 8		*A. baumannii* — 4		*Campylobacter* spp. — 3		*Staphylococcus* spp. — 7		*Enterococcus* spp. — 7		*C. pseudodiphtheriticum* — 1	
		n	SC	*n*	SC	*n*	SC	*n*	SC	*n*	SC	*n*	SC	*n*	SC	*n*	SC	*n*	SC
Imipenem	MHB-Obolensk	14	S	2	S	1	S	8	I	4	S	–	–	–	–	7	I	–	–
	MHB-BD	14	S	2	S	1	S	8	I	4	S	–	–	–	–	7	I	–	–
Meropenem	MHB-Obolensk	14	S	2	S	1	S	8	S	4	S	–	–	–	–	–	–	–	–
	MHB-BD	14	S	2	S	1	S	8	S	4	S	–	–	–	–	–	–	–	–
Ceftazidime	MHB-Obolensk	1 13	R S	2	S	1	S	8	I	–	–	–	–	–	–	–	–	–	–
	MHB-BD	1 13	R S	2	S	1	S	8	I	–	–	–	–	–	–	–	–	–	–
Levofloxacin	MHB-Obolensk	14	S	2	S	1	S	8	I	4	S	–	–	7	I	7	S	–	–
	MHB-BD	14	S	2	S	1	S	8	I	4	S	–	–	7	I	7	S	–	–
Ciprofloxacin	MHB-Obolensk	1 13	R S	1 1	R S	1	S	8	I	4	I	1 2	R I	7	I	7	S	1	R
	MHB-BD	1 13	R S	1 1	R S	1	S	8	I	4	I	1 2	R I	7	I	7	S	1	R
Linezolid	MHB-Obolensk	–	–	–	–	–	–	–	–	–	–	–	–	7	S	7	S	1	S
	MHB-BD	–	–	–	–	–	–	–	–	–	–	–	–	7	S	7	S	1	S
Vancomycin	MHB-Obolensk	–	–	–	–	–	–	–	–	–	–	–	–	7	S	3 4	R S	1	S
	MHB-BD	–	–	–	–	–	–	–	–	–	–	–	–	7	S	3 4	R S	1	S
Ampicillin	MHB-Obolensk	2 12	R S	2	S	1	S	–	–	–	–	–	–	–	–	7	S	–	–
	MHB-BD	2 12	R S	2	S	1	S	–	–	–	–	–	–	–	–	7	S	–	–
Colistin	MHB-Obolensk	14	S	1 1	R S	1	S	1 7	R S	4	S	–	–	–	–	–	–	–	–
	MHB-BD	14	S	1 1	R S	1	S	1 7	R S	4	S	–	–	–	–	–	–	–	–
Tetracycline	MHB-Obolensk	–	–	–	–	–	–	–	–	–	–	1 2	R S	1 6	R S	–	–	1	S
	MHB-BD	–	–	–	–	–	–	–	–	–	–	1 2	R S	1 6	R S	–	–	1	S
Gentamicin	MHB-Obolensk	3 11	R S	1 1	R S	1 1	R R	–	–	3 1	R S	–	–	3 4	R S	–	–	–	–
	MHB-BD	3 11	R S	1 1	R S	1 1	R R	–	–	3 1	R S	–	–	3 4	R S	–	–	–	–
Erythromycin	MHB-Obolensk	–	–	–	–	–	–	–	–	–	–	1 2	R S	1 6	R S	–	–	–	–
	MHB-BD	–	–	–	–	–	–	–	–	–	–	1 2	R S	1 6	R S	–	–	–	–
Tigecycline	MHB-Obolensk	–	–	2	S	–	–	–	–	–	–	–	–	7	S	3 4	R S	–	–
	MHB-BD	–	–	2	S	–	–	–	–	–	–	–	–	7	S	3 4	R S	–	–
Trimethoprim-sulfamethoxazole	MHB-Obolensk	14	S	2	S	1	S	–	–	4	R	–	–	3 4	R S	–	–	–	–
	MHB-BD	14	S	2	S	1	S	–	–	4	R	–	–	3 4	R S	–	–	–	–

Note. *n* — number of strains; SC — sensitivity category. A dash — testing for this antibiotic has not been performed; S — sensitive to standard dosing regimen; R — resistant; I — sensitive to increased exposure to antibiotic.

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