RESPONSE

of official opponent for the dissertation work

Wu Lin

on the topic «Resistance factors of bacterial nosocomial infections causative agents as background for the modern antimicrobials development»,

presented for obtaining the degree of Doctor of Philosophy

in the field of knowledge 09 Biology

in the specialty 091 Biology

Relevance of the dissertation topic.

A significant part of pharmaceutical developments in Ukraine and in the world concerns new drugs or generic drugs for the treatment of infectious diseases. Such diseases cause numerous complications, and severe infectious diseases cause fatal consequences, which requires a constant search for more effective antimicrobial substances.

Serious infectious diseases that are difficult to treat are often caused by pathogens of nosocomial infections - pathogens that spread in hospitals and acquire increased resistance, adapting to widely used antimicrobial agents. Up to 10% of all infectious diseases in the world are caused by nosocomial infections, which the patient receives while staying in medical institutions. Therefore, the attention of the developers of antimicrobial agents is drawn, first of all, to the means of combating resistant pathogens representing nosocomial infections.

The assortment of antimicrobial substances in modern pharmacy mainly includes antibiotics of various origins, chemical substances of various nature and enzyme preparations, and in modern medicinal preparations they try to combine them to increase efficiency. However, one of the most important tasks on the way to creating new antimicrobial agents is the use of substances that can overcome the adaptive mechanisms of pathogens and prevent the emergence of even more resistant forms of them. It is possible to implement this task primarily by studying the mechanisms and factors of resistance of pathogens, and therefore to determine the most sensitive targets for the action of antiseptics.

Such tasks and systemic approaches to solving the problems of overcoming the resistance of nosocomial infectious agents were chosen in the presented dissertation work of Wu Lin, which determines the relevance of the topic from a scientific and practical point of view.

Evaluation of the validity of the scientific results of the dissertation, their reliability and novelty.

The scientific novelty of the results of the dissertation research is as follows:

- the resistance factors of the isolated *Bacteroides thetaiotaomicron* DSMZ 2079 strain, which can be targets for the development of antimicrobial agents, were established, namely the presence of four homologues of the self-transmitted conjugative transposon CTnDOT, which ensures the expansion of resistance to tetracycline and erythromycin, and the antibiotic resistance genes *rpoB* and *tuff* were identify;
- based on the results of the analysis of the isolated *Pseudomonas oryzihabitans* JN 873340 strain and its comparison with 29 other strains of the species, the possibility of quick identification of its geographical origin based on two hypervariable regions V4 and V5 of the 16S rRNA sequence was shown, and therefore the possibility of quick selection of an effective agent of antimicrobial therapy;
- the minimum inhibitory concentrations of the new streptofungin antibiotic against a number of test cultures of pathogens were established, as well as the absence of toxicity in the range of effective concentrations, which determines its potential as an active pharmaceutical ingredient;
- the possibility of modification of *Pichia pastoris* yeast cells for use as a substrate for wide screening of sortase A inhibitors – effective antimicrobial substances against gram-positive pathogens – is shown.

The main provisions and conclusions of the dissertation work are well-argued, based on the obtained experimental results and are in accordance with modern theoretical provisions and data highlighted in the literature review. The reliability of the work results is confirmed by statistical data processing using modern software and analysis using bioinformatics databases. The validity and reliability of the results and conclusions is also based on their approval at scientific conferences.

So, the scientific task set in the dissertation regarding the identification of approaches and new ways related to the creation of modern antimicrobial drugs against bacterial pathogens of nosocomial infections based on the study of their resistance factors has been completely fulfilled, and the applicant has fully mastered the methodology of scientific activity.

Assessment of the content of the dissertation, its completeness and compliance with the principles of academic integrity.

According to its content, the dissertation work of the applicant Wu Lin fully complies with the Standard of Higher Education in the specialty 091 Biology and research directions in accordance with the Applied Biology educational program.

The dissertation is a completed scientific work and indicates the presence of the applicant's personal contribution to the scientific field of applied biology.

After examining the similarity report based on the results of checking the dissertation for text matches, it can be concluded that Wu Lin's dissertation is the result of independent research by the acquirer and does not contain elements of falsification, compilation, fabrication, plagiarism and borrowing. Used ideas, results and texts of other authors have proper references to the corresponding source.

Language and style of presentation of results

The dissertation is written in English.

The dissertation has a clear and accessible scientific style of presentation of results while maintaining a logical sequence. The work uses the generally accepted scientific terminology of the field and the structure of the presentation of the work.

The dissertation consists of an introduction, 5 chapters, conclusions, a list of references and annexes. The total volume of the dissertation is 144 pages.

In the introduction, the relevance of the topic and its connection with the subject of the university are substantiated, the goal and tasks of the research are formulated, the scientific novelty of the results and their practical significance are shown, the personal contribution of the author and the approbation of the work are revealed.

The literature review is written competently, with proper knowledge of the problem, generalization of experimental data and conclusions about the prospects of practical use of research results. The presented materials contain a sufficiently complete and critical coverage of the current state of knowledge about modern approaches to the study of pathogens of nosocomial infections and directions for the development of the novel antiseptics. Most of the publications listed in the bibliography were published after 2010.

Chapter 2 describes research materials and methods according to the stages of the work, namely the selection and analysis of selected pathogens of infectious diseases, molecular genetic methods of analyzing their characteristics, analysis of selected substances for the development of antimicrobial compositions, etc. The list

of used modern microbiological, biochemical, molecular genetic, statistical studies meets the set objectives and indicates the adequacy of the obtained experimental data.

The results of the author's research and their discussion are presented in chapters 3-5. In chapter 3 "Research and control of the distribution and resistance factors of the bacterial nosocomial infections causative agents", the author conducts the study and analysis of selected clinical strains of pathogens of the genera *Acinetobacter, Pseudomonas and Bacteroides*. The risk groups for *A.baumannii* infection, the dynamics of the spread of the pathogen in different departments of the clinic and its stability depending on the frequency of disinfection in the institution are determined. A decrease in the sensitivity of clinical strains of *A.baumannii* to third-generation cephalosporins (up to 55-77%), as well as the effectiveness of monitoring the sensitivity of nosocomial infection and the selection of appropriate antimicrobial agents, is shown.

A molecular genetic analysis of the isolated strain *P. oryzihabitans* JN 873340 was carried out and the possibility of identifying its geographical origin by two hypervariable regions V4 and V5 was shown. This can be used to quickly identify and take into account the characteristics of strains of different origins when choosing an effective antimicrobial agent for treatment, which is important to do in the first phases of the process.

The genome of the isolated clinical strain *B. thetaiotaomicron* DSMZ 2079 was sequenced and annotated, showing that it has a one- and two-component system for recognizing environmental signals, four homologues of the self-transmitted conjugative transposon CTnDOT, which provides the extension of resistance to tetracycline and erythromycin. The genes rpoB and tuf (in loci JHR92_RS03155 and JHR92_RS03195, respectively) were identified, which determine the resistance of the strain to antibiotics, and therefore can be chosen as targets in the development of antimicrobial agents against this pathogen.

Chapter 4 "Screening and characterization of new antimicrobial substances" is devoted to the study of new promising substances for the development of antimicrobial agents and methods of their selection. The prospects of finding new antibiotics among streptomycetes and the established characteristics of the antibiotic streptofungin isolated from *Streptomyces albus* 2435 are shown. Antifungal activity against C. albicans ATCC 10231 at low antibiotic concentrations (10 μg/ml) and absence of toxicity over a wide range of concentrations (from 2.5 to 500 μg/ml) are important. This determines the prospects of this antibiotic as a separate antifungal agent, as well as a drug of accompanying therapy in the treatment of bacterial nosocomial infections.

The presented research showed the possibility of modifying the yeast cells of *Pichia pastoris* and using them instead of expensive synthetic substrates for the screening of inhibitors of Sortase A. This was used to develop a new method for the broad screening of inhibitors of Sortase A, which catalyzes the processes of fixation of Gram-positive bacteria on body cells, and therefore is an ideal target for new antimicrobial agents, for example against staphylococcal infection.

The studies presented in Chapter 5 "Proposals and recommendations regarding the development of promising antimicrobials" highlight the processes of developing a ready-made form of a combined antiseptic based on the studied antibiotic streptofungin and a bacteriolytic enzyme synthesized simultaneously by the producer *Streptomyces albus*. The mechanism and result of the antimicrobial action of the enzyme on test cultures (staphylococcus and *Escherichia coli* cells) are shown with the presentation of microphotographs illustrating the destruction of the cell wall of the cultures. The proposed pharmaceutical composition of an antiseptic ointment based on these antimicrobial substances, which in combination will be aimed at overcoming such an important mechanism of pathogen resistance as biofilm.

The section also presents Recommendations developed based on the results of research and analysis of the literature on the implementation of resistance monitoring of nosocomial infections and the principles of development of modern antimicrobial drugs.

The dissertation was prepared in accordance with the requirements of the order of the Ministry of Education and Culture of Ukraine dated January 12, 2017 No. 40 "On approval of requirements for the preparation of the dissertation".

Publication of the results of the dissertation work

The scientific results of the dissertation are highlighted in 10 scientific publications of the applicant, including: 4 articles in periodical scientific publications indexed in the Web of Science Core Collection and/or Scopus databases, of which 2 articles in publications classified in the first — third quartiles (Q1— Q3) according to the classification of SCImago Journal and Country Rank or Journal Citation Reports, 4 articles in specialized foreign periodicals.

Also, the results of the dissertation were tested at 3 scientific professional conferences, and the conducted research passed a bioethical examination and received a conclusion that the work was carried out in compliance with the principles of bioethics and can be recommended for submission to the defense for the purpose of obtaining a scientific degree. Thus, the scientific results described in the dissertation are fully covered in the applicant's publications.

Disadvantages and comments to the dissertation work.

1. Since the composition of the ointment based on the studied active substances is proposed in the work, it would be desirable to highlight the modern principles of creating dosage forms and selecting excipients in the literature review.

2. Why *S. aureus* was not chosen as one of the pathogens for research, which is one of the most common agents of nosocomial infections, and some of the strains have multiple resistance?

3. In the scientific novelty, it should also be noted the established principle of modification of *Pichia pastoris* yeast cells, which was later used by the author to obtain a substrate for a wide screening of sortase A inhibitors.

4. It is not entirely clear why the strain S. albus 2435 (p. 90) was used for the biosynthesis of the antibiotic streptofungin, if its antagonistic activity was not the highest among others (table 4.1)?

5. Why were two different types of test cells (MDBK and A549) chosen when studying the toxicity of the new antibiotic streptofungin, and how was this taken into account in the obtained results?

6. In conclusion 8, it would be desirable to indicate the composition of the proposed pharmaceutical composition of the ointment and indicate the effective concentrations of the studied antimicrobial substances (the enzyme cytal and the antibiotic streptofungin).

I believe that the comments expressed are not decisive and do not reduce the general scientific novelty and practical significance of the results and do not affect the positive evaluation of the dissertation work.

Conclusion on the dissertation work

I believe that the dissertation work of degree of Doctor of Philosophy candidate Wu Lin on the topic "Resistance factors of bacterial nosocomial infections causative agents as background for the modern antimicrobials development" is performed at a high scientific level, does not violate the principles of academic integrity and is a completed scientific study, a set of theoretical and the practical results of which solve a scientific task that is of significant importance for the field of knowledge 09 – Biology. The dissertation work in terms of relevance, practical value

and scientific novelty fully meets the requirements of the current legislation of Ukraine, which are provided for in clauses 6 - 9 of the "Procedure for awarding the degree of Doctor of Philosophy and annulment of the decision of the one-time specialized academic council of the institution of higher education, scientific institution on awarding the degree of Doctor of Philosophy" , approved by Resolution No. 44 of the Cabinet of Ministers of Ukraine dated January 12, 2022.

The applicant Wu Lin deserves to be awarded the degree of Doctor of Philosophy in the field of knowledge 09 - Biology with the specialty 091 - Biology.

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"ХАРКІВСЬКИЙ ПОЛІТЕХНІЧНИЙ ІНСТИТУТ"
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