

## REVIEW

for 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*

Infectious diseases significantly affect a human's quality of life and can become a long-term problem, and severe infections can lead to fatal consequences. The ongoing military operations in Ukraine and the world add urgency to the fight against infected wounds and the spread of infections. One of the most difficult to treat are nosocomial infections, which spread in the center of infected patients in hospitals and hospitals, because their pathogens acquire increased resistance to the antimicrobial agents used.

That is why the main efforts of the developers of antimicrobial drugs are aimed at the fight against the pathogens of nosocomial infections - the search for new, more effective substances and the creation of combined drugs with different mechanisms of action. For such developments, it is important to choose a target in the cell of the pathogen and the mechanism of influence, which would exclude or minimize the possibility of changes in the pathogen and the appearance of new means of protection.

The basis of effective development of antimicrobial agents is a detailed study of the characteristics of individual pathogens and the establishment of their resistance and pathogenicity factors, which are obviously targets for modern antiseptics. Therefore, the topic of the presented dissertation work by Wu Lin, which is devoted to the analysis of the characteristics of bacterial pathogens of nosocomial infections and the justification of systemic approaches to the development of effective antimicrobial agents, is important and relevant.

### *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 are:

- determination of two hypervariable regions (V4 and V5) of the 16S rRNA sequence of the isolated clinical strain *Pseudomonas oryzihabitans* JN 873340, and

therefore the possibility of rapid identification of its geographical origin and rapid selection of an effective therapeutic agent already at the first stage of therapy;

- establishment of minimum inhibitory concentrations of a new antibiotic of microbial origin (streptofungin) against a number of pathogens, as well as the absence of toxicity in the range of effective concentrations;

- identification of antibiotic resistance genes (rpoB and tuf) of the isolated *Bacteroides thetaiotaomicron* DSMZ 2079 strain and establishment of its resistance mechanisms due to four homologues of the self-transmitted conjugative transposon CTnDOT, which ensures the expansion of resistance to tetracycline and erythromycin.

The validity and reliability of the results and conclusions is confirmed by discussion at several international scientific conferences, as well as by the use of modern software, bioinformatics databases and statistical processing of experimental data. The main provisions and conclusions of the dissertation work are based on the obtained experimental results and their analysis. The dissertation was completed as part of research work (state registration number 0112U002390) at the Department of Industrial Biotechnology and Biopharmacy of Igor Sikorskyi KPI and within the framework of several projects (No. 817324, No. 201513) in the laboratory of environmental ecology and research of health problems at Hainan Medical University, Haikou (China).

Therefore, it can be noted that the scientific task set in the dissertation to establish the principles of development of modern antimicrobial drugs based on the analysis of resistance factors of bacterial pathogens of nosocomial infections has been completely fulfilled, and the recipient 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.

The similarity report based on the results of checking the dissertation for text matches indicates that Wu Lin's dissertation is the result of the applicant's independent research 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 work is written in an understandable scientific style, with a standard structure, laid out in an accessible logical sequence using generally accepted scientific terminology. The dissertation consists of an introduction, a literature review, research materials and methods, three sections of own research and their discussion, conclusions, a list of literature with 210 sources and appendices. The total volume of the dissertation is 144 pages.

In the introduction, the relevance of the topic and its connection with the scientific topics of the department are properly substantiated, the purpose 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 shown.

The literature review shows the current state of the problem on the topic of the dissertation, which provides modern approaches to the study of pathogens of nosocomial infections and directions for the development of the latest antiseptics: the presented information is summarized and conclusions are drawn about promising areas of research. The sources of information are modern and relevant.

The second chapter (Materials and methods of research) presents the characteristics of the strains and materials used, the methods of analysis of selected pathogens of infectious diseases, molecular genetic methods of analysis of their characteristics, etc. The used research methods are modern and correspond to the set tasks, which confirms the adequacy of the obtained results.

Chapter 3 "Research and control of the distribution and resistance factors of the bacterial nosocomial infections causative agents" presents the results of the study of isolated strains of pathogens of the genera *Bacteroides*, *Acinetobacter*, and *Pseudomonas*.

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) and, therefore, the possibility of choosing an effective antimicrobial agent was shown. The genome of the selected clinical strain *B. thetaiotaomicron* DSMZ 2079 was sequenced and annotated, the *rpoB* and *tuf* genes were identified, which determine the strain's resistance to antibiotics, and therefore can be selected as targets in the development of antimicrobial agents against this pathogen.

A reduction in the sensitivity of clinical strains of *Acinetobacter baumannii* to third-generation cephalosporins, the effectiveness of the internal system for monitoring the sensitivity of nosocomial infection and identified risk groups for *A. baumannii* infection, the dynamics of the spread of the pathogen in different departments of the clinic and its stability are shown.

Section 4 shows the development of a screening method and the possibility of finding new promising antibiotics of microbial origin. A method of modifying *Pichia pastoris* yeast cells and using them for screening Sortase A inhibitors instead of expensive synthetic substrates has been developed; developed a method of broad screening of inhibitors of Sortase A, which catalyzes the fixation of gram-positive bacteria (for example, staphylococcus) on body cells, determining pathogenesis.

The high antifungal activity of the new antibiotic streptofungin from *Streptomyces albus* 2435 in low concentrations (up to 10 µg/ml) was established, as well as the absence of toxicity in the effective range, which determines its prospects as an antimicrobial substance.

In chapter 5 "Proposals and recommendations regarding the development of promising antimicrobials" the processes of developing a ready-made form of a combined antiseptic (in the form of an ointment) based on the studied antibiotic streptofungin and an antimicrobial enzyme are investigated, the mechanism and result of the enzyme's antimicrobial effect on test cultures are shown, a pharmaceutical composition is developed antiseptic ointment based on these antimicrobial substances. Recommendations on the implementation of resistance monitoring of nosocomial infections and the principles of development of modern antimicrobial drugs have been developed.

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 covered in 10 scientific publications of the applicant, including: 4 articles in periodical scientific publications indexed in the Scopus database, of which 2 articles in publications assigned to the second quartile (Q2) according to the SCImago Journal and Country Rank or Journal Citation classification Reports, 4 articles in specialized foreign periodicals. The results of the dissertation are also presented in 2 published abstracts of reports at scientific and practical conferences.

The dissertation passed a bioethical examination, which certified that it was carried out in compliance with the principles of bioethics and was recommended for

submission to the defense. Thus, the scientific results presented in the dissertation are fully covered in the scientific publications of the applicant.

### ***Disadvantages and comments to the dissertation work***

1. Bioinformatics analysis methods are widely used in the dissertation, so it would be desirable to provide examples of similar studies in the literature review and to indicate why these databases (CAZy, KEGG) were chosen.

2. In the materials and methods, it would be worthwhile to describe in more detail the methods of statistical processing of experimental data used in the work (paragraph 2.2.14).

3. Why was a representative of the genus *Bacteroides* chosen as one of the pathogens for the study, which is not among the common representatives of the causative agents of nosocomial infections?

4. When developing a screening method for inhibitors of sortase A, two methods of determining its activity were studied - flow cytometry and fluorescence spectrophotometry, which were recognized as sensitive. However, which of the methods was ultimately chosen in the implemented screening method and why?

5. The results of the study of the antimicrobial effect of the proposed combined ointment (Table 5.1) show the maximum activity against staphylococcus (65% degradation), and it is much lower against other specified pathogens. Will such a tool be effective as a result?

6. Paragraph 4.1 contains too detailed a description of the existing approaches to the screening of sortase A inhibitors and the rationale for the research plan, which would be better presented in a literature review.

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.

**Reviewer:**

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