REVIEW

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of the materials submitted for participation in the competition for the academic position of "Associate Professor" at UP in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3 Biological Sciences

(Microbiology - Microbial Pathogenesis)

1. General Introduction of the Received Materials

By order РД-21-332 dated 15/02/2023 of the Rector of "Paisii Hilendarski" University of Plovdiv (UP), I have been appointed a member of the scientific jury of the competition for the academic position of "Associate Professor" at UP in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3 Biological Sciences (Microbiology - Microbial Pathogenesis), published in the State Gazette, No. 92 of 18/11/2022 for the needs of the Department of Biochemistry and Microbiology at the Faculty of Biology (FB). Only one candidate has submitted documents for participation in the announced competition, namely Chief Asst. Dr. Mariana Marhova-Koseva from the same Department. The set of materials submitted by the candidate on paper and in electronic format complies with The Regulations for the Development of the Academic Staff of UP (RDASUP) and includes the following documents:

- Application form to the Rector of UP for admission to the competition;
- CV in European format;
- Master's degree of higher education;
- Educational and scientific doctoral degree;
- List of scientific publications;
- Copies and citations of scientific publications;
- Reference for the fulfilment of the minimum national requirements stipulated in The Act on the Development of the Academic Staff in the Republic of Bulgaria (ADASRB)
- Reference for the fulfilment of the specific requirements of FB, according to RDASUP;
- Annotations of materials;
- Reference by the author for contributions to the scientific publications;
- Declaration of originality and absence of plagiarism;

– Other documents, including evidence of academic publications and work experience.

For her participation in the competition, Chief Asst. Dr. Marhova has submitted an authored monograph, 35 scientific publications, one manual for laboratory exercises and one textbook, which do not duplicate the materials from the dissertation for the obtaining of a scientific and educational doctoral degree, therefore, all submitted materials are accepted for evaluation.

The content of the documents submitted allows me to categorically define the procedure as lawful.

2. Brief Biographical Information About the Candidate

In 1985, Mariana Marhova obtained a Master's degree in Biology from the Faculty of Biology at P. Hilendarski UP. In the period 1986-1990 she was a full-time PhD student in the Institute of Biochemistry and Physiology of Microorganisms at the Academy of Sciences of the USSR and in 1991 she obtained a doctoral degree (Candidate of Biological Sciences) in the scientific specialty of biochemistry. Dr. Marhova's academic career at BF at UP began in 1991, initially as a part-time assistant in Genetics, Microbial Genetics and Immunology and in 1993 she was elected a full-time assistant in the same disciplines. From 1994 to 1997, she occupied the academic position of a Senior Assistant and since 1997 she has been a Chief Assistant in the Department of Biochemistry and Microbiology of FB at UP, where she teaches lecture courses and laboratory exercises in a wide range of disciplines.

The candidate's creative biography is entirely within the scope of the announced competition and is related to the faculty and core unit that have stated the need for this competition.

3. General Description of the Candidate's Activities

Evaluation of the Candidate's Scientific Activity

Group of indicators A: Indicator 1 Dissertation for the acquiring of a Doctoral degree - requirement of The Act on the Development of the Academic Staff in the Republic of Bulgaria (ADASRB) - 50 pts., fulfilment by the candidate - 50 pts.

Group of indicators B (Sum of indicators 3 or 4):

Indicator 3 Habilitation dissertation - monograph or Indicator 4 Scientific articles in publications that are referred and indexed in the Scopus and Web of Science global databases of scientific information - requirement of ADASRB - 100 pts., fulfilment by the candidate - 100 pts.

For her participation in the competition and in satisfaction of indicator B, Chief Asst. Dr. Marhova has submitted a monographic paper entitled ***"Drug resistance and virulence in Enterobacterales uropathogens. Studies in the period 1997 - 2021".***

According to the World Health Organization, one of the most serious problems of modern society is the dramatically increasing number of resistant strains isolated from patients, which poses serious challenges to clinical practice. On the other hand, bacteria of the *Enterobacteriaceae* family associated with urinary tract infections stand out among the most frequent causative agents of recurrent infections. The fact that the rapid development of molecular biological methods of identification has led to intense dynamics in the taxonomic status of microorganisms, particularly pronounced in the *Enterobacteriaceae* family, should not be overlooked. The accumulation of all these factors puts research related to the determination of drug resistance of uropathogens of the *Enterobacterales* order at the centre of scientific interest of modern clinical microbiology. In this line of thought, the monographic work submitted for the competition entitled "***Drug resistance and virulence in Enterobacterales uropathogens. Research in the period 1997 - 2021***" is particularly relevant and useful because it is dedicated to an important problem both from a theoretical and practical point of view. The monograph has 159 standard A4 pages and is structured into seven sections: Introduction, Urobiome and Urinary Tract Infections, Etiological Structure of Urinary Tract Infections (UTI), Drug Resistance in *Enterobacterales* order uropathogens, Virulence in *Enterobacterales*, Summary and References. The Introduction poses the problem of heterogeneity of the *Enterobacteriaceae* family and defines the current taxonomic status of the newly introduced *Enterobacterales* order. In this initial section of the monograph, the author already clearly states that the studies on drug resistance and virulence of the representatives of the order are provoked by the needs of the clinical practice and, in this sense, the results of such research would have an important applied contribution. The second section of the monograph critically analyses the current protocols for clinical microbiological examination of urine and their shortcomings, which negatively affect the accuracy of the obtained results. Different molecular biological methods are also discussed, which the author proposes to be included as an element of updated complex protocols for identification of microbial isolates from the urinary tract to allow correct determination of their species affiliation with a sufficient degree of discriminability. The section describes urinary tract infections (UTIs) in detail and highlights their degree of societal importance. The third section of the monograph contains an in-depth analysis by the author of the etiological structure of UTIs of hospitalized and ambulatory patients in the period 2001-2018, based on the statistical data from BulStar. Increased species diversity of isolates was reported with *Escherichia coli* being the dominant species followed by *Proteus mirabilis, Klebsiella pneumoniae and Enterococcus faecalis.* The personal studies of Chief Asst. Dr. Marhova are even more extensive in scope; they cover a 24-year period and include more than 6,000 strains from urine samples from GBIMDL "ZINVEST-K" Ltd. Plovdiv, MDL Cibalab Ltd - Sofia and IMDL "Chronolab" - Plovdiv (now IMDL "Synevo Bulgaria" Ltd) and MMA - Plovdiv (Military Hospital). The most dominant species among the

isolates is *E. coli,* followed by *P. mirabilis, K. pneumoniae* and *K. oxytoca, Serratia* and the species *Morganella morganii, Enterobacter cloacae, Citrobacter diversus* and *C. freundii*. The fourth section of the monograph discusses drug resistance in uropathogens of the *Enterobacterales* order, describing the mechanisms of emergence and transfer of antibiotic resistance. Following the analysis of the literature data on the subject, the author encloses the results of a considerable amount of her personal research, which includes a huge number of strains and different classes of antibiotics. The results of this research are presented, analysed and discussed in detail and a tendency towards a significant increase in the number of UTI-causing strains resistant to penicillins, fluoroquinolones, sulfamethoxazole/trimethoprim and, to a much lesser extent, to second and third generation cephalosporins and aminoglycosides is found. *E. coli* strains synthesizing broad-spectrum beta-lactamases, as well as strains with multidrug resistance are identified. A strain of *K. pneumoniae subsp. pneumoniae*, demonstrating resistance to all tested antibiotics except meropenem and imipenem, which was confirmed by the National Reference Laboratory for Control and Monitoring of Antibiotic Resistance at the National Centre of Infectious and Parasitic Diseases in Sofia is identified and defined as having a "broad (extended) resistance" (XDR). At the beginning of the section *"Virulence in Enterobacterales"* the author emphasizes the heterogeneity of the order and its importance as a causative agent of various human infections. The different pathotypes of *Enterobacterales* are described with emphasis on *E. coli*. Virulence factors – fimbriae and adhesins, toxins, siderophores, motility, methods to avoid the body's immune defences, and serum resistance are discussed in detail. The monograph focuses on bacterial communities and biofilm formation - genetic determinants, stages of biofilm formation, antimicrobial resistance and biofilm resistance. The section, similar to the other sections of the monograph, includes a large body of personal research related to serotyping, determination of adhesins and haemolysins production and their genetic determinants, morphotypes and serum resistance of UPEC. A substantial part of the results in the monograph is dedicated to the determination of the biofilm-forming capacity of *Enterobacterales* representatives associated with UTI. Drug resistance of biofilms to four antibiotics from different groups is compared with that of planktonic cells of the same species. The virulence characteristics of uropathogens were found to be highly specific, and additional risks in UTI therapy and prophylaxis were found to be associated with subtherapeutic levels of commonly used antimicrobials. The last section of the monograph is a summary, which compares the authors' personal results with data published in BulStar and formulates the main conclusions of this large-scale research, which has allowed the creation of an etiological map of urogenital tract infections in ambulatory patients. There are reported dynamics in the proportion of different *Enterobacterales* species as causative agents of UTIs, which highlights the need for coordinated actions by diagnostic laboratories and regulatory authorities to disclose such data in order to reduce the incidence of empiricism in the management of this type of infection. The shortcomings of current protocols in clinical

microbiology laboratories for the establishment of causative agents of UTIs leading to "false negative results" are demonstrated and the need for their updating is strongly articulated. The relatively high levels of antibiotic resistance reported, as well as the regional and national disparities found, strongly argue against empirical treatment of community-acquired UTIs. Differences in resistance levels by region requite the development of a system to monitor antibiotic resistance at both regional and national levels through the improvement of the National BulStar Programme. The literature review is impressive, comprehensive and topical, based on 458 literature sources, more than 80% of which are from the last 10-15 years.

In conclusion, I believe that the presented monographic work represents a comprehensive and sufficiently representative in its scope and time interval research on uropathogens of the *Enterobacterales* order, which can definitely claim originality at a national level. The manner of formulation of the author's personal results and their interpretation against the background of a thorough literature review indicate a high level of competence in the field of the studied problem.

Group of indicators D (Sum of indicators 5 to 10) – requirement of ADASRB - 200 pts., fulfilment by the candidate – 211 pts.

Indicator 7 Scientific publications in journals that are referred and indexed in the Scopus and Web of Science global databases of scientific information.

To cover this group of indicators, Chief Asst. Dr. Marhova presents 15 scientific publications, distributed by quartiles as follows: 2 publications in journals of the Q_2 quartile (2 x 20 = 40 pts.); 5 publications in journals of the Q_3 quartile (5 x 15 = 75 pts.) and 8 publications in journals of the Q_4 quartile (8 x 12 = 96 pts.).

The publications can be grouped into two main research areas - (1) microbial pathogenesis, which is the main research field of Chief Asst. Dr. Marhova (publications from D.1 to D.5) and (2) ecology of microorganisms (publications from D.6 to D.15), an area where she skilfully used her experience from the field of microbial pathogenesis - the determination of biofilm-forming capacity and drug resistance to microbial isolates from water bodies, wetlands and soils in order to assess the degree of anthropogenic impact on different habitats.

By analysing the conclusions of the monographic work and the results in publications D.1 to D.5, in the field of microbial pathogenesis, I can highlight the following major contributions:

- ✓ This is the first time a representative, large-scale and original study of the etiological structure of urogenital infections in ambulatory patients in the city of Plovdiv over a 24-year period was conducted in the country, and an etiological map of urinary tract infections in Southern Bulgaria was compiled in correlation with the data from BulStar and the National Centre of Infectious and Parasitic Diseases - a contribution of scientific and applied nature.
- ✓ The need for changing and updating current diagnostic protocols used in the evaluation of

urogenital tract infections, which were introduced in the 50-60s of the 20th century, in order to provide a more accurate and reliable diagnosis, is proved in a definitive way - a contribution of an applied nature.

- ✓ A systematic evaluation of the drug susceptibility of a large number of isolates from ambulatory patient samples (6,330 strains from microbiological examination of urine and 267 strains from genital swabs) revealed increasing resistance to fluoroquinolone products, widespread prevalence of ESBL-producing strains as well as such with multiple resistance - a finding of scientific application.
- ✓ The identified high levels of antibiotic resistance characterised by great regional and national variations strongly motivates the need for the development and implementation of an effective system to monitor antibiotic resistance through the improvement of the National BulStar Programme - a contribution of an applied nature.
- ✓ The presence of plasmid-mediated quinolone resistance has been demonstrated and the importance of the adhesive potential of bacteria as a tool in urinary tract pathogenesis has been confirmed. Biofilm formation and serum resistance were identified as significant factors in bacterial adaptation to the urinary tract - a contribution of scientific interest.
- ✓ An improved experimental protocol for the evaluation of serum resistance of *Enterobacterales* isolates has been developed, which could be easily applied in the clinical practice related to the diagnostics of UTIs - a contribution of methodological and applied nature.

By analysing the results in publications D.6 to D.15 in the field of microorganism ecology, I can highlight the following major contributions:

- ✓ This is the first time when the applicability of microbiological indicators as quality indicators in ecological assessment of complex reservoirs has been proven in Bulgaria. A nationally adapted classification system for water quality assessment according to the number of the sanitary indicators total number of heterotrophic microorganisms (TVC), *Escherichia coli*, fecal coliforms (FC) and fecal streptococci (FS) was developed - a contribution of methodological and scientific applied nature.
- ✓ This is the first time when a metagenomic analysis of the microbial community structure was performed in the country via massive parallel sequencing of the gene encoding 16S rRNA in complex and economically important reservoirs, which is a necessary step for the clarification of the microbial ecology of reservoirs - a contribution of scientific nature.
- ✓ This is the first time the ecological potential of economically important reservoirs has been determined according to the information available by the Basin Directorates (BD) and the performed direct monitoring of the water bodies. Specific values for the ecological capacity

for fish production in net cages have been proposed for each of the studied complex reservoirs, which have been adopted by the Ministry of Environment and Water as borderline for the respective reservoir when issuing or renewing permits for the use of surface waters for the purposes of aquaculture and related activities - a contribution of scientific and applied nature.

- ✓ This is the first time when the spatial variation and physiological diversity of soil microbial communities in natural wetlands and the cultivated rice fields in the Maritza River basin, protected under the Birds Directive 2009/147/EC as natural habitats, have been investigated - a contribution with a scientific nature.

I define the contributions of Chief Asst. Dr. Marhova in the scientific publications as significant, scientifically applied and methodological, with a high degree of originality, as well as complementing and building upon existing knowledge.

Group of indicators E (Sum of the points in indicator 11) - requirement of ADASRB - 50 pts., fulfilment of the candidate - 132 pts.

Chief Asst. Dr. Marhova has presented a list including 66 independent positive citations in the *Scopus* and *Web of Science* system, without self-citations.

Evaluation of the Candidate's Teaching and Pedagogical Activity

Chief Asst. Dr. Marhova has 30 years of teaching experience, and for the last 5 years she has delivered 2,804 lectures, equated to exercises and 1,732 hours of laboratory exercises in both the B.Sc. and M.Sc. programmes, which means that in the B.Sc. programme alone she had an average workload of 580 hours annually where the annual norm is 360 hours. The range of disciplines Chief Asst. Dr. Marhova holds lecture courses in is impressive: Immunology, Microbial Pathogenesis, Microbial Genetics, Immunogenetics, Plasmids, Genetics of Industrial Microorganisms, Clinical Microbiology, Biology of Infectious Diseases. The laboratory practicums developed for all the disciplines mentioned above are her personal contribution, except for the discipline Immunology - developed jointly with a team from the department. Chief Asst. Dr. Marhova is a co-author of the Manual for Laboratory Exercises in Microbiology, published by a team of the Department of Biochemistry and Microbiology and a co-author of a textbook on "Biological Membranes", published by a team of the Faculty of Biology at UP. The 23 successful graduates are a reflection of her teaching experience, skill and willingness to work with students. The candidate was the second supervisor of a successfully graduated Ph.D. student - Marinela Tsankova, whose dissertation topic was entirely in the research field of Chief Asst. Dr. Marhova. The candidate in this competition is the author of 12 curricula (5 in the B. Sc. and 7 in the M. Sc. programme) in basic and specialized disciplines: Immunology, Microbial Pathogenesis, Microbial Genetics, Medical Microbiology,

Clinical Microbiology, etc., which are included in the teaching documentation of all successfully accredited specialties in professional fields 4.3 Biological Sciences and 5.11 Biotechnology in the Faculty of Biology at UP.

The teaching experience of Chief Asst. Dr. Marhova is unquestionable, and the overall teaching and pedagogical activity of the candidate is at a level that guarantees quality education of the students.

Additional requirements have been introduced for the academic position "Associate Professor" in the Faculty of Biology at UP, that were fulfilled by Chief Asst. Dr. Marhova as follows:

- ✓ The candidate must be the author or co-author of at least 2 teaching aids in the relevant field and specialty of the announced competition – fulfilment: 2 teaching aids;
- ✓ The candidate must have supervised at least 5 successful graduates in the relevant field of the competition – fulfilment: 23 graduates and 1/2 Ph. D. student;
- ✓ The candidate must provide evidence of at least 5 years of teaching experience – fulfilment: 29 years, 9 months and 3 days.
- ✓ The candidate must have been a participant in at least 2 scientific projects – fulfilment: 8 projects, 2 of which were projects of the Scientific Research Fund at the Ministry of Education, 1 - a National Scientific Programme project, 1 - international and 4 – internal for the institution.
- ✓ The candidate must have organizational and administrative experience (the last 5 years) in the faculty – Chief Asst. Dr. Marhova was a member of the faculty committee for faculty certification, faculty quality assurance committee, member of the faculty teams that prepared the self-evaluation reports for different accreditation procedures, organizing committees of student competitions in FB, promotion of students, etc.

All additional requirements of FB for the academic position of "Associate Professor" are fulfilled by Chief Asst. Dr. Marhova in a categorical manner.

4. Evaluation of the Personal Contributions of the Candidate

I believe that the personal contribution of Chief Asst. Dr. Marhova to the realisation of the publications submitted for the competition is relevant to the level of her competence.

5. Critical Comments and Recommendations

I have no critical comments to make on the documents submitted.

6. Personal Impressions

I have known Mariana Marhova since 2005 and I consider my impressions to be objective. She is very well prepared theoretically in the field of research and teaching, precise in her experimental work, responsible to the tasks she is given, business-like and honest in her relations. I believe that she is respected by all colleagues and students at FB.

CONCLUSION

The documents and materials presented by Chief Asst. Dr. Mariana Ivanova Marhova - Koseva meet the formal and substantive requirements of ADASRB, the Implementing Rules of ADASRB and RDASUP.

The candidate in the competition has presented a sufficient number of scientific works, published after the materials used in the defence of her PhD degree, highlighting original scientific, scientific and applied, and applied contributions, which enrich the existing knowledge. The results achieved by Chief Asst. Dr. Marhova in terms of teaching and scientific research activities fully meet the specific additional requirements of FB at UP for the academic position of "Associate Professor".

After my familiarisation with the materials and scientific publications presented in the competition, the analysis of their significance and the scientific and applied contributions in them, I find it justified to give my positive evaluation and to recommend to the Scientific Jury to prepare a proposal report to the Faculty Council of the Faculty of Biology for the election of Chief Asst. Dr. Mariana Ivanova Marhova - Koseva for the academic position of "Associate Professor" at the Faculty of Biology in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3 Biological Sciences (Microbiology - Microbial Pathogenesis).

17/03/2023 Reviewer: .....

(Prof. Dr. Velizar Gotchev)