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**ANNOTATION**

dissertation work for the degree of Doctor of Philosophy in the specialty: 6D110100 "Medicine"

**Topic: «Antibiotic resistance and clonal structure**

**of clinical isolates *of Acinetobacter baumannii***

**in Central Kazakhstan**"

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**Relevance of the topic:**

Hospital (nosocomial) infections (NIs) are a major health problem worldwide [1].

The frequency of NI development varies quite widely and depends on the region, the profile of the hospital, and anti-epidemic measures. According to WHO, about 8.7 per cent of hospitalized patients develop NI, i.e. about 1.4 million people worldwide suffer from hospital-acquired infections [2]. Analysis of the official statistics of the Ministry of Health of the Republic of Kazakhstan (MH RK) showed that the incidence of NI is 2.1-2.38 per 100 thousand cases of hospitalization [3]. It is possible that the official statistics on NI incidence in our country, as in the Russian Federation and other countries of the former Commonwealth of Independent States, do not reflect the real situation [4]. Several authors agree that more than 1/3 of NI contamination can be prevented [5, 6].

The etiological spectrum of NI pathogens depends on the profile of each particular department or hospital. For example, in maternity institutions, as well as in surgical departments, gram–positive microorganisms, in particular, Staphylococcus aureus, have been the main etiological agent in the recent past, while in urological departments - gram-negative flora

Since the beginning of the 2000s, an increase in the incidence of acinetobacter infections has been observed in many countries of the world, accompanied by a rapid spread of antimicrobial resistance of pathogens [7]. Due to the increased mortality from A. baumannii infections, as well as limited antibiotic treatment options, in 2017, WHO designated carbapenem-resistant A. baumannii (carbapenem-resistant *Acinetobacter baumannii* – CRAB) as a high-priority target for research and development of new antibiotics [8]. *A. baumannii* has been classified as a problematic pathogen by the Infectious Diseases Society of America (IDSA) [9].

According to N. M. Bisenova et al., in the Republic of Kazakhstan, non-fermenting microorganisms, including *A. baumannii*, are more often isolated from patients in intensive care units of multidisciplinary hospitals *A. baumannii* [10, 11].

NI caused by A. baumannii is characterized by an endemic course, while epidemiology suggests the presence of a large number of related cases caused by strains with common characteristics that are genetically homogeneous, which suggests a clonal nature.

According to literature data, nosocomial *A. baumannii* belongs to three well-known clonal lines that form the world-famous epidemic clones (CC1, CC2, and CC3). These international clonal lines are responsible for the majority of hospital cases of acinetobacter infections and are characterized by antibiotic resistance to clinically important antimicrobial drugs [12].

In addition, modern hospital strains *of A. baumannii* are characterized by the presence of carbapenemases: *blaOXA-23,*  *blaOXA-24/40, blaOXA-58* [13], including the species*-specific blaOXA-51*. In recent years, there have been reports of the release of GES-5 beta-lactamases, which also have carbapenemase activity [13]. A number of studies conducted in neighboring countries [14, 15] demonstrate the threat of widespread spread of such strains in the Republic of Kazakhstan (RK) and the Central Asian region. The number of local clonal complexes also increases annually.

The above arguments dictate the need for research to obtain current data on the frequency of development of Ni, the etiological spectrum of pathogens, the level of resistance of nosocomial isolates of *A. baumannii* and clonal lines in Central Kazakhstan.

**The aim of the study** is a prospective multicenter microbiological study of antibiotic resistance, epidemiological characteristics, and clonal structure of carbapenemase-producing *A. baumannii* isolates in Central Kazakhstan.

To achieve the chosen goal, the following tasks were formulated:

*Objective 1.* To assess the prevalence *of A. baumannii* in the structure of nosocomial infections in multidisciplinary hospitals in Central Kazakhstan*.*

*Objective 2*. Based on a multicenter study, determine the sensitivity *of A. baumannii* isolates isolated from patients with nosocomial infections to antimicrobial drugs.

*Objective 3.* To evaluate the role of carbapenemases of various classes in the formation of resistance of nosocomial *A. baumannii* isolates to beta-lactam antibiotics.

*Objective 4.* To assess the role of international clones of high epidemic risk *A. baumannii* in the formation of the etiological role of nosocomial infections in multidisciplinary hospitals in Central Kazakhstan.

**Scientific novelty**

For the first time, *A. baumannii* has been shown to colonize ICU patients, mainly in the upper and lower respiratory tracts.

For the first time, data on the sensitivity of strains collected in the framework of multicenter research to antimicrobial drugs, obtained by reference method of serial microdilution in the broth Mueller - Hinton, were presented.

For the first time local data on epidemiology in Central Kazakhstan were presented for clinical isolates *A. baumannii*, producers of carbopenemasis *blaOXA-23* and *blaOXA-58 .*

SNP-types *of A. baumannii* were first identified in Central Kazakhstan.

For the first time, data on the distribution and circulation *А. baumannii* of high-risk international *A. baumannii* clones CG208(92)OXF/CG2PAS and CG231(109)OXF/CG1PAS in Kazakhstan hospitals were obtained.

For the first time, it was established that the distribution *of blaOXA-58 producers* is exclusively associated with isolates of the CG184OX/CG218PAS clonal complex.

**The main provisions submitted for defense:**

1. In the etiological structure of nosocomial infections, an important role is played by *A. baumannii* isolates isolated in the dominant number from patients in intensive care units (ICU).
2. The etiological structure of nosocomial infections in hospitalized patients includes *A. baumannii,* isolates that are resistant to aminoglycosides, fluoroquinolones, and carbapenems, with MDR and XDR resistance profiles.
3. The resistance of hospital isolates of A. baumannii, isolated in large multidisciplinary hospitals of Central Kazakhstan, to carbapenemas is due to the production of *blaOXA-23* and *blaOXA-58* carbapenemas. Carbapenemase producers have associated resistance to most non-betalatactam antibiotics.
4. The largest number *of A. baumannii* isolates in the study was assigned to SNP-type 8 and SNP-type 16.
5. Carbapenem resistance observed in the bacterial population *of A. baumannii* is associated with the international clones of high epidemic risk CG208 (92)OXF/CG2PAS and CG231(109) OXF/CG1PAS.
6. The distribution *of blaOXA-58* producersis exclusively associated with isolates of the CG184OX/CG218PAS clonal complex.

**Practical significance**

The obtained data on sensitivity to antimicrobial drugs are used in the work of hospitals for rational use and justification of the purchase of antibacterial drugs.

The methods used in this work are used to study the local epidemiological structure of nosocomial infections caused *by A. baumannii.* It is necessary to plan and implement anti-epidemic measures in healthcare organizations of the Republic of Kazakhstan aimed at curbing the resistance *of A. baumanii*, which are producers of blaOXA-23 and blaOXA-58.

The obtained data on the clonal structure *of A. baumanii* in Central Kazakhstan are used by practical healthcare in order to assess, treat and prevent infections caused by this pathogen.

The antibiotic-resistant *A. baumanii* (MDR, XDR) obtained as a result of the study, deposited in the laboratory of the Non-Commercial Joint-Stock Company «Medical University of Karaganda» (NCJSC MUK), can be used as reference strains for further studies of antibiotic resistance in the Republic of Kazakhstan.

The data obtained as part of a prospective multicenter microbiological study are used in shaping national policies as part of a roadmap for curbing antibiotic resistance at the national level.

**Personal contribution of the author**

The author was directly involved in the analysis and generalization of literature data, organization of a set of materials, and conducting all stages of microbiological and molecular genetic studies. The author independently collected and processed the material, analyzed, summarized the research results and their description, wrote and designed all the chapters of the dissertation work. The materials of the dissertation work were processed and analyzed personally by the author in the amount of 95%.

**Implementation in practice**

Based on the materials of the dissertation, 1 certificate of registration of rights to the object of copyright No. 40188 dated 06.11.2023 «Distribution of international clones of high epidemic risk CG208(92)OXF/CG2PAS, CG231(109)OXF/CG1PAS and CG184OX/CG218PAS in the *Auinetobacter baumannii* in Central Kazakhstan» was obtained. A. Turmukhambetova, I. A. Kadyrova, D. A. Klyuev (Appendix A). There are acts of implementation of the results of research work in the practical and scientific activities of the NCJSC MUK clinic and the NCJSC MUK research laboratory (Appendix B).

**Approbation of the work:**

The main provisions and results of the dissertation work were presented at:

* The 6th International Congress of the Kazakhstan Association of Medical Laboratory Diagnostics during the work of the Section «Modern interdisciplinary and integral technologies in laboratory medicine - early diagnosis, antibiotic resistance and laboratory control of infectious diseases» (oral report «Global and local approaches to the problem of antibiotic resistance» (April 19-20, 2018, Almaty, Kazakhstan);
* The second republican forum of specialists of laboratory medicine of the Republic of Kazakhstan Laboratory practice. Look to the future» (oral report «Antibiotic resistance of pathogens of urinary tract infections in Kazakhstan» December 7, 2018, Nur-Sultan, RK);
* 54th Congress of the European Society for Surgical Research (oral report «» March 13-15, 2019, Geneva, Switzerland);
* Conference-seminar «Laboratory diagnostics and monitoring of treatment of infectious diseases» (oral report «Practical aspects of application of MALDI-TOF mass spectrometry in clinical microbiology and scientific research» (October 3-4, 2019, Almaty, Kazakhstan);
* Online conference «Week of antimicrobial therapy and clinical microbiology» (oral report «Antibiotic resistance in Kazakhstan», (September 7-13, 2020, Moscow, Russia);
* Online conference of the III Republican Forum of Laboratory Medicine Specialists of the Republic of Kazakhstan Laboratory Practice. Look to the future» (oral report «What you need to know about secondary infection at COVID-19» 23 October 2020, Nur-Sultan, Kazakhstan);
* Interdisciplinary online conference «Non-communicable and infectious diseases during the COVID-19 pandemic: new reality, mistakes, lessons, experience» (oral report «Secondary infection at COVID-19», November 12-13, 2020, Russian Federation/RK);
* Microbiology Society Annual Conference Online 2021, has been awarded the Journal of Medical Microbiology «Best Oral Presentation Prize» for the presentation «Respiratory pathogens co-infection in patients with COVID-19 pneumonia in Kazakhstan»;
* II Kazakhstan congress of infectious disease «Infectious diseases in the context of globalization: challenges and solutions» (oral report «Antibiotic resistance. Past. Present. Future», October 7-8, 2021, Astana, Kazakhstan);
* III Kazakhstan Congress of Infectious Diseases with International Participation «Infectious Diseases in the Context of Globalization: Challenges and Solutions» (oral report «Problems of Antibiotic Resistance in Hospitals of Kazakhstan», October 5-6, 2023, Astana, Kazakhstan);
* Expanded meeting of the Institute of Life Sciences of NCJSC MUK, (October 23, 2023, Karaganda, RK)

**Publications**

Based on the materials of the dissertation, 7 articles and 2 theses were published in Russian, Kazakh and English, including 3 articles – in publications recommended by the Committee for Quality Assurance in Science and Higher Education of the Ministry of Education and Science of the Republic of Kazakhstan, 4 articles and 2 theses-in publications with a non-zero Impact Factor and included in the Scopus database: 2 publications in international publications included in Q2 of the Scopus information database (66% and 74% at the time of publication), 1 publication included in Q1 of the Scopus information database (60% at the time of publication), 1 publication included in Q4 of the Scopus information database (20% at the time of publication). The work was tested during 4 international conferences, 7 republican conferences with international participation and at an extended meeting of the Institute of Life Sciences of the NJSC MUK.

**Structure and scope of the dissertation:**

The dissertation contains 83 pages of typewritten text, consists of an introduction, a literature review, the main part (materials and methods of research, chapters of own research), conclusions, conclusions, practical recommendations, 3 tables, 30 figures and a list of references that includes 3 sources, 294 applications.

**Materials and methods of research:**

The study included isolates obtained from large multidisciplinary hospitals in Karaganda, Astana and Zhezkazgan, isolated during 2011-2019 from inpatient patients with a confirmed infection that developed 48 hours after hospitalization. Isolation and primary identification of bacterial isolates were performed in local laboratories using standard microbiological methods [16]. Further, the strains were transferred to a shared Laboratory in accordance with standard biosafety procedures [17] NJSC MUK (Karaganda), where the final identification of all bacterial isolates was carried out. Part of the molecular genetic studies on SNPtyping was performed at the Research Institute of Antimicrobial Chemotherapy (NII AH) in Smolensk (Russia).

378 isolates *A. baumannii* were studied, as well as 13 control strains from the American Type Culture Collection and 8 control strains from the collection of the Research Institute of Agricultural Sciences (Smolensk).

The isolates were identified by matrix-associated laser desorption / ionization-time-of-flight mass spectrometry (MALDI-TOF MS). The species identification of *A. baumannii* isolates was confirmed by detecting the genes of species-specific beta-lactamases of the blaOXA-51 group by real-time PCR.

Sensitivity to AMP was determined by the disco-diffusion method on Muller-Hinton agar and by serial micro-dilution in Muller-Hinton broth. Sensitivity to AMP was determined for 224 *A. baumannii* strains.

The results of antimicrobial sensitivity testing were interpreted in accordance with the EUCAST v 11.0 criteria [18].

Primary phenotypic screening of MBL products was performed using double disks with ethylene diamine tetraacetate [19]. Carbapenemase activity was detected in a modified Hodge test and by carbapenem inactivation method[20].

The presence of acquired Class D carbapenemase genes common to *Acinetobacter spp.* (blaOXA-23, blaOXA-24/40, and blaOXA-58 groups), as well as Class B carbapenemase (MBL) of VIM, IMP, and NDM groups was determined by real-time PCR. Strains of *A. baumannii* (blaOXA-40, blaOXA-51)*, A. pittii* (blaOXA-40, blaOXA-58)and *P. aegidinosa* (VIM, IMP, NDM) carrying known carbapenemase genes were used as positive controls. The results of antibiotic sensitivity assessment and determination of carbapenemase genes were deposited as a project in the AMRcloud database [21] for further processing.

To assess the genetic diversity *of A. b aumannii strainsaumannii* применяли метод , the SNP-typing method was used. Detection of each SNP was performed by allele-specific real-time PCR in accordance with the high-throughput approach proposed by Myakishev et al. [22].

The selected set of 21 MLST SNP locus provided a comparison between the obtained SNP types with known STs and CCs in accordance with the MLST nomenclature MLST, including the so-called «high-risk international clones». The correspondence between SNP typing and MLST data was provided by the SQL database and the software platform [23]. The SNPTAb database was used to store SNP typing data with data on individual isolates (e.g., source, geographic origin, isolation data, carbapenem resistance, and carbapenemase production).

Statistical processing. The initial data analysis was carried out in the Whonet 2022 program, with the calculation of CI. Statistical processing was performed using the onlineplatform AMRcloud [21], which is developed in the programming language «R». Descriptive analysis with calculation of absolute and relative frequencies, median values, and confidence intervals using the Wilson method was applied to the data. Categorical variables were compared using Fisher's exact test and Holm's multiple comparison correction. To analyze the data and visualize the typing process*, A. baumannii* used the goeBURST tool in the PHYLOViZ 2.0 program [24], which uses plug-ins to process large data sets both by the number of samples and by loci. PHYLOViZ includes the ability to integrate and visualize molecular epidemiological data, in our case by SNP. PHYLOViZ includes the ability to implement hierarchical clustering methods. At each stage of the algorithm, one pair of clusters is selected that meets the minimum dissimilarity criterion. The selected clusters are combined, and in the next step, a new cluster is considered that corresponds to their combination.

**Conclusions:**

*A. baumannii* plays an important role in the etiology of NI in hospitalized patients. Infections associated with *A. baumannii*,were diagnosed in 8.71% (n=378) of cases from 2011 to 2019. Of the isolated *A. baumannii* strains, 60% were obtained from ICU patients, 25.93% - from surgical patients.

In the majority of cases*, A. baumannii* was isolated from respiratory samples: 16.40% – from the nasopharynx, 15.61% – from the trachea, and 11.65% - from sputum. In the ICU*, A. baumannii* was detected most often (16.67%) from the trachea, in 11.84% of cases – from the wound and catheter, in 10.96% – from sputum and nasopharynx.

*A. baumannii'*s resistance to carbapenems (imipenem and meropenem) was 81.25% and 78.57%, respectively, to amikacin, gentamicin and ciprofloxacin – 79.91%, 65.47% and 89.29%, respectively. MDR strains accounted for 87.89% of *A. baumannii* (95%CI 82.96-91.54). XDR strains were obtained in 18.39% (95%CI 13.85-23.99). No pan-resistant strains were detected. The sensitivity of the isolates to colistin and tigecycline was preserved.

The following class D carbapenemase genes were identified in 82.14% of *A. baumannii* isolates: blaOXA-23 (78,57%) and blaOXA-58 (3,57%).The isolated CRABs were resistant to ciprofloxacin (97.28%, 95%CI), amikacin (89.67%) and gentamicin (69.57%). That is, resistance to carbapenems in *A. baumannii* hospital isolates isolated in large multidisciplinary hospitals in Central Kazakhstan is due to the production of carbapenemases blaOXA-23 and blaOXA-58. Carbapenemase producers had associated resistance to most non-betalactam antibiotics.

The largest number of *A. baumannii* isolates were assigned to SNP-type 8 (69.64%) and SNP-type 16 (5.36%). The spread of carbapenem-resistant strains of *A. baumannii* in Central Kazakhstan is associated with international clones of high epidemic risk CG208(92)OXF/CG2PAS (80.8%) and less frequently CG231(109)OXF/CG1PAS (1.79%).

The spread of blaOXA-58 producers (SNP-type 83) is associated exclusively with isolates of the CG184OX clonal complex/CG218PAS.

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