Ministry of Health of the Republic of Moldova

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Manuscript
U.D.C: [616.98:579.861.2]-022.3-036.22 (478) (043.2)

BUGA Diana

EPIDEMIOLOGY OF NOSOCOMIAL INFECTIONS WITH METHICILLIN-RESISTANT *STAPHYLOCOCCUS* IN THE REPUBLIC OF MOLDOVA

331.01 – EPIDEMIOLOGY

Summary of the doctoral thesis in medical sciences

Chișinău, 2021

The thesis was elaborated at the Scientific Laboratory "Nosocomial Infections", the Department of Epidemiology, the Department of Preventive Medicine, *Nicolae Testemitanu* State University of Medicine and Pharmacy in the Republic of Moldova

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The defense will take place on 27.01.2022, 14:00 o'clock within "Nicolae Testemitanu" SUMPh, 165 Ștefan cel Mare şi Sfânt str, office 205 in the meeting of the Commission for public defense of the doctoral thesis, approved by the decision of the Scientific Consortium Council of 02.12.2021 *(minutes no. 25).*

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Contents

LIST OF ABBREVIATIONS

AM	–Antimicrobial drugs
AMR	– Antimicrobial resistance
EARSNet	– European Antimicrobial Resistance Surveillance Network
EEA	– European Economic Area
ESBL	–Extended spectrum beta-lactamases
CA-MRS	– Community-acquired methicillin-resistant *Staphylococcus*
DHC	– District Health Centers
ECDC	– European Center for Disease Prevention and Control
HA-MRS	– Healthcare-acquired Methicillin-resistant *Staphylococcus aureus*
HCAI	– Healthcare-associated infections
PHCS	–Public healthcare setting
IEM	– Institute of Emergency Medicine
PSIs	– Purulent-septic infections
MRS	– Methicillin-resistant *Staphylococcus*
MSS	– Methicillin-susceptible *Staphylococcus*
MRSA	– Methicillin-resistant *Staphylococcus aureus*
MSSA	– Methicillin- susceptible *Staphylococcus aureus*
MH	– Ministry of Health
WHO	– World Health Organization
RCH	– Republican Clinical Hospital „Timofei Moșneaga”
CPS	– Coagulase-positive staphylococci
CNS	– Coagulase-negative staphylococci
EU	– European Union
ICU	– Intensive care units
VISA	– Vancomycin-intermediate *Staphylococcus aureus*
VRSA	– Vancomycin-resistant *Staphylococcus aureus*

INTRODUCTION

The novelty of the subject. Healthcare-associated infections (HAIs) have become one of the major public health problems from the clinical, epidemiological and socio-economic perspectives. They represent a special chapter of pathology highlighted in the context of modern medical advances, determined by the diversity of diagnostic and therapeutic maneuvers, also due to the irrational use of antibiotics, especially the broad-spectrum ones, which leads to the selection of resistant bacteria [1, 2, 3, 4, 5].

Healthcare-associated infections caused by antibiotic-resistant microorganisms are one of the most important challenges for modern medicine today. A large part of *Staphylococcus aureus* infections are produced by a microbial pathogen resistant to a wide range of antibiotics used in the hospital environment, namely, methicillin-resistant *Staphylococcus aureus* (MRSA) [6].

From the first cases of MRSA to date, the problem has increased dramatically, the incidence of MRSA infections reaching endemic proportions in some hospitals. In Europe, a north-south gradient is observed, MRSA strains being rare in Scandinavian hospitals (<2%) and much more widespread in hospitals in Mediterranean countries (> 40%) [7, 8, 9].

Although in recent years the percentage of methicillin-resistant *Staphylococcus aureus* strains has decreased in the European Union / European Economic Area (EU / EEA), from 19.6% in 2014 to 16.9% in 2017, however, MRSA remains an important challenge at the European level, with high MRSA levels in several countries and an increased overall antimicrobial resistance [10, 11, 12, 13].

In 2017, the World Health Organization (WHO) established the most important categories of multidrug-resistant germs**,** which required the introduction of new therapeutic options with 3 priority levels: critical, high and medium; methicillin-resistant *Staphylococcus aureus* strains belonging to the high level [14, 15].

Shortly after the emergence of MRSA, it was realized that the problem of methicillin resistance involves not only coagulase-positive staphylococci (CPS), but also coagulase-negative staphylococci (CNS). Although in the beginning, these species of microorganisms were given little importance, being considered part of the normal flora of the skin and nasal mucosa, today, due to their resistance to antibacterial preparations, they have become a source of concern for the medical system. They are especially associated with the use of foreign bodies, implants, etc., which are indispensable in modern medicine [16, 17]. Increased rates of antibiotic resistance have been shown to be even a greater problem for CNS than for *Staphylococcus aureus*, limiting the treatment options [18, 19].

Methicillin resistance has been shown to have a negative impact on clinical and economic outcomes, especially in terms of increased morbidity and mortality and long-term hospitalization, as well as the need for additional interventions to mitigate the clinical impact [20, 21, 22, 23, 24, 25].

The ranking of staphylococci first in the etiology of bacterial infections, the annual increase in the number of methicillin-resistant staphylococcal strains and the emergence of strains resistant to spare antistaphylococcal antibiotics, place this medical condition among emerging infectious diseases [26, 27, 28, 29].

In the Republic of Moldova, studies on the incidence of infections caused by methicillin-resistant *Staphylococcus* (MRS) are fragmentary [28, 29, 30].

Knowledge of local epidemiology of methicillin-resistant *Staphylococcus* infections will contribute to raising the awareness of the problem and implementation of rational measures for surveillance and control of the given medical condition.

Purpose

To study the epidemiology of nosocomial infections caused by methicillin-resistant *Staphylococcus* in the Republic of Moldova in order to optimize the surveillance and control system.

Research objectives

1. To determine the share of purulent-septic infections (PSIs) with methicillin-resistant *Staphylococcus* in the Republic of Moldova, based on general hospitals, maternity wards and healthcare settings in rural areas, and to carry out a comparative analysis.

2. To determine the epidemiological peculiarities, as well as the risk factors in purulent-septic infections caused by MRS.

3. To determine the degree of susceptibility/resistance of MRS strains isolated from patients with purulent-septic infections depending on the group and type of antibiotic.

4. To determine the medical and socio-economic impact of purulent-septic infections with MRS.

Scientific novelty

Studies on PSI epidemiology caused by MRS have been carried out internationally. At the national level, for the first time a complex epidemiological study was conducted on the epidemiology of PSIs caused by MRS and the level of spread was determined.

Theoretical significance and applied value of the work:

1. The rate of PSIs caused by MRS has become known, as well as the epidemiological peculiarities depending on the profile of inpatient departments in the Republic of Moldova.

2. The risk factors and the socio-economic impact of the PSIs produced by MRS were determined.

3. The degree of sensitivity / resistance of the MRS strains was determined, depending on the group and type of antibiotic.

4. Measures were proposed to optimize the surveillance and control of PSIs caused by MRS in healthcare settings.

Practical implementation of results

1. The research results were implemented within the Department of Preventive Medicine, Discipline of Epidemiology, as teaching material for graduate and postgraduate training of doctors as well as in the scientific laboratory " Intrahospital infections"

2. Two scientific-practical seminars on "The problem of spread and antibiotic resistance of nosocomial infections with methicillin-resistant *Staphylococcus*" were organized within the Public Health Care Setting (PHCS) of the Republican Clinical Hospital "Timofei Moşneaga" (03.02.2021) and PHCS Emergency Medicine (19.02.2021).

3. The study results will complete the next edition of the "Guide to surveillance and control of nosocomial infections".

4. The results obtained were presented in scientific papers at 16 scientific forums, as well as in 15 scientific publications.

Approval of results

- National Scientific Conference with international participation “Optimizing epidemiological surveillance in nosocomial infections”, October 13-14, 2017, Chisinau, Republic of Moldova.
- Annual scientific conferences of *Nicolae Testemitanu* SUMPh students and teaching staff, 2018, 2019, 2021 Chisinau, Republic of Moldova.

- Всероссийская научно-практическая конференция специалистов по контролю ИСМП "Инфекции, связанные с оказанием медицинской помощи - междисциплинарный подход к профилактике" с международным участием, 24-25 апреля 2019, Екатеринбург.
- The scientific event dedicated to the U.M.Ph days in Craiova, the XLIXth edition, June 7-8, 2019, Craiova, Romania.
- The VIIIth Congress of specialists in the field of public health and health management, October 24-25, 2019, Chisinau, Republic of Moldova.
- The XII National Conference on Microbiology and Epidemiology "Romanian Microbiology and Epidemiology - Achievements, Evolutions and Perspectives", November 14-16, 2019, Bucharest, Romania.
- International Congress of Students and Young Research Doctors "MedEspera-2020", 8th edition, September 24-26, 2020, Chisinau, Republic of Moldova.
- Ежегодная всероссийская научно-практическая конференция с международным участием "Актуальные проблемы эпидемиологии инфекционных и неинфекционных болезней 2020", 21-23 октября 2020, Москва.
- Congress dedicated to the 75th Anniversary of the *Nicolae Testemitanu* SUMPh founding, October 20-23, 2020, Chisinau, Republic of Moldova.
- The European Researchers' Night 2020, Chisinau, November 27, 2020.
- The scientific-practical seminar “The problem of spread and antibiotic resistance of nosocomial infections with *Acinetobacter* and methicillin-resistant *Staphylococcus*” within the PHCS of the Republican Clinical Hospital “Timofei Moșneaga”, February 3, 2021.
- The scientific-practical seminar “The problem of spread and antibiotic resistance of nosocomial infections with *Acinetobacter* and methicillin-resistant *Staphylococcus*” within PHCS Institute of Emergency Medicine, February 19, 2021.
- National Scientific Conference with International Participation “Infectious Diseases in the Modern World: Challenges and Perspectives”, March 26, 2021, Chisinau, Republic of Moldova.

The thesis was discussed, approved and recommended for defense at the joint meeting of the academic staff of the Discipline of Epidemiology and the Scientific Laboratory "Intrahospital Infections" of 14.09.21 (minutes no. 3), at the meeting of the Scientific Seminar 331. Public Health, 333 Occupational health and biomedicine / 331.01. Epidemiology, 331.02. Hygiene, 333.01 Occupational hygiene, of 26.10.21 (minutes no. 4), and finally, within the Consortium Council of *Nicolae Testemitanu* State University of Medicine and Pharmacy of 02.12.2021 (minutes no. 25).

Research methodology and study design were evaluated and approved by *Nicolae Testemitanu* SUMPh Research Ethics Committee, no. 46 of 12.04.2018.

Publications

Based on the thesis materials, 15 scientific papers were published, of which, 5 in impact factor journals and 4 articles in national journals, including 2 single-authored articles, as well as 6 abstracts at national and international scientific conferences.

Subdivisions and institutions within which the research was conducted

The research was carried out in the Scientific Laboratory "Intrahospital Infections" under the program: 15.817.04.03F "Study on nosocomial infections with *Acinetobacter*, *Pseudomonas* and methicillin-resistant *Staphylococcus* between 2015 and 2018", based on bacteriological investigations of patients with purulent-septic infections from two healthcare settings (PHCS Institute of Emergency Medicine, PHCS Republican Clinical Hospital "Timofei Moşneaga"), a maternity ward (PHCS Municipal Clinical Hospital "Gheorghe Paladi"), as well as rural healthcare settings (on the model of three districts: Criuleni, Strașeni and Ialoveni, bacteriological investigations being carried out in the bacteriology laboratory within the Public Health Center, Chisinau).

Keywords: Healthcare-associated infections, purulent-septic infections, methicillin-resistant *Staphylococcus*, methicillin-susceptible *Staphylococcus*, clinical forms, antibiotic resistance, multi-drug resistance, risk factors, therapeutic management, socio-economic impact.

RESEARCH METHODOLOGY

General characteristics of the research group

The study was performed based on the retrospective descriptive epidemiological study of the results of bacteriological investigations of patients with purulent-septic infections caused by methicillin-resistant *Staphylococcus* from two healthcare settings (PHCS Republican Clinical Hospital "Timofei Moșneaga" (inpatient department no. 1) and PHCS Institute of Emergency Medicine (inpatient department no. 2) for a period of 4 years (2014-2017), the share of MRS strains in the multiannual dynamics being determined for the period 2014-2019; of patients from healthcare settings in rural areas (on the model of three districts, Criuleni, Ialoveni, Straseni) and maternity wards (maternity ward within the PHCS of the Municipal Clinical Hospital "Gheorghe Paladi"), 2017.

A total of 13,219 staphylococcal strains were analyzed, 11,803 being isolated from healthcare settings, 1,090 from rural areas and 326 from maternity wards. Methicillin-resistance of *Staphylococcus* strains was established depending on the resistance to oxacillin (2014-2017) and cefoxitin (2018-2019), which was determined by using the disc-diffusion test (Kirby-Bauer) and the automatic microbiological analyzer VITEK 2 Compact in the healthcare settings. Only the disc-diffusion test was used in the PHCS Municipal Clinical Hospital "Gheorghe Paladi" and in the rural environment. The results were interpreted according to the standard recommendations of CLSI (Clinical and Laboratory Standards Institute) and EUCAST (European Committee on Antimicrobial Susceptibility Testing).

In order to determine the risk factors, the antibacterial management and the socio-economic impact, the data from the patient's medical records with PSIs caused by methicillin-resistant staphylococci were analyzed. The basic group was made up of patients with bacteriological investigations positive for methicillin-resistant *Staphylococcus* (MRS), while the control group comprised patients with positive bacteriological investigations for methicillin-sensitive *Staphylococcus* (MSS). The study included all patients with septicemia, trophic ulcers, inflammation, and lung damage, in whom staphylococcus was isolated, hospitalized in the RCH during 2014-2017.

In this context, 462 patients' medical records were retrospectively analyzed, 269 medical records of patients with nosocomial purulent-septic infections with MRS and 193 medical records of patients with nosocomial purulent-septic infections with MSS.

In order to achieve the purpose and objectives set, the general design of the research has been elaborated, which is presented in figure 1.

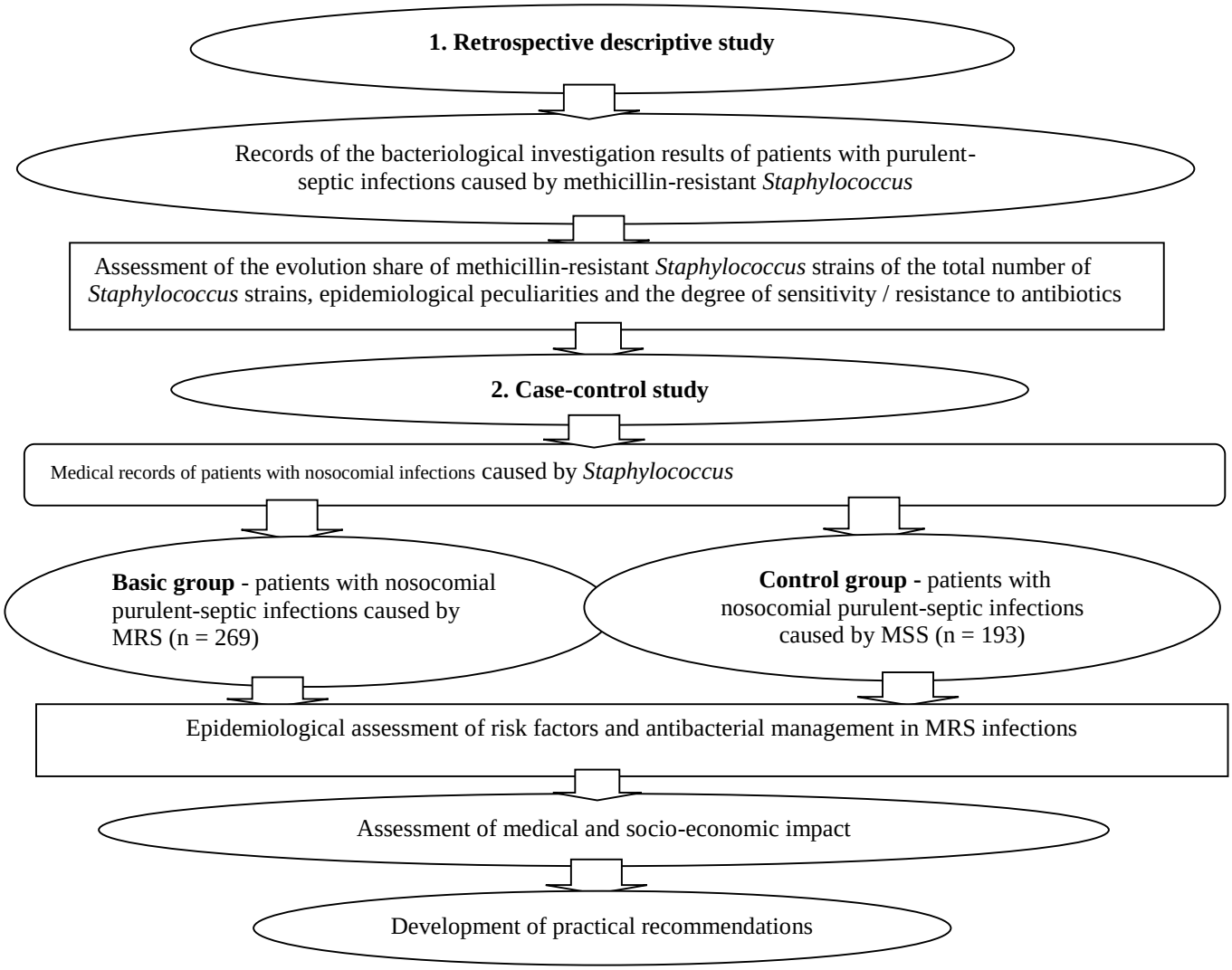


Figure 1. **Research design**

Research methods applied in the study

Microbiological methods

Staphylococcal strains were isolated, as well as antibiotic sensitivity / resistance was determined in the medical laboratories under study, using the classical method, described by Galeţchi P. et al. (1997), Buiuc D. (2009), and the automated system VITEK 2 Compact (bioMérieux) [31, 32, 33].

Epidemiological methods

The epidemiological method was used to study and to describe the morbidity of purulent-septic infections caused by MRS, as well as to calculate the degree of antibiotic sensitivity / resistance, antibacterial management, risk factors and socio-economic impact. The determination

of epidemiological features and the degree of antibiotic susceptibility / resistance of MRS infections refers to descriptive observational epidemiological studies and is based on the method of retrospective analysis according to the methodology described by Prisacari Viorel in "General Epidemiology. Bases of evidence-based medicine" (2020) [34].

Criteria for inclusion in the study:

- ✓ all bacteriological investigations positive for MRS and MSS examined in the bacteriological laboratories of inpatient departments included in the study;

Exclusion criteria from the study:

- ✓ bacteriological investigations negative for MRS and MSS.

The socio-economic impact and risk factors were determined in a retrospective case-control study, based on data from patients` medical records with PSIs caused by MRS, being compared with data from the medical records of patients with PSIs caused by MSS.

The research groups were made according to the method described in "General Epidemiology. Bases of evidence-based medicine" (2020), patients with MRS being the exponents of the basic group, while patients with MSS constituted the control group.

Statistical methods of data processing

The results obtained were entered into electronic databases and statistically processed, using the computer programs WHONET 5.6, Microsoft Office Excel 2010 and IMB SPSS Statistics 22.

SYNTHESIS OF THESIS CHAPTERS

1. EPIDEMIOLOGY OF NOSOCOMIAL INFECTIONS WITH METHICILLIN-RESISTANT *STAPHYLOCOCCUS* (LITERATURE REVIEW)

Chapter I comprises the synthesis of the research results on MRS infections exposed in the literature, structured in 4 subchapters: the situation of healthcare associated infections with methicillin-resistant *Staphylococcus* worldwide, clinical and epidemiological aspects of MRS infections, antibiotic-resistance of MRS strains, risk factors and preventive measures in MRS infections. The analysis of the literature on the topic argues the need for this study.

2. RESEARCH MATERIAL AND METHODS

Chapter II contains information on the research methodology.

3. EPIDEMIOLOGY OF PURULENT-SEPTIC INFECTIONS WITH METHICILLIN-RESISTANT *STAPHYLOCOCCUS* IN THE REPUBLIC OF MOLDOVA

3.1. Spread of purulent-septic infections with methicillin-resistant *Staphylococcus* in healthcare settings

The incidence of *Staphylococcus* strains in the etiology of PSIs in healthcare settings according to the study results makes up - 24.2%.

During 2014 – 2019, in the two healthcare settings, under the study, 11,803 staphylococcal strains were isolated from patients with purulent-septic infections, of which 10,861 (92.0%), were tested to susceptibility / resistance to oxacillin / cefoxitin. As a result, it was found that the share of methicillin-resistant *Staphylococcus* strains in healthcare settings is on average 40.5% (95 % CI 39.6-41.4) (table 1).

Table 1. **Share of MRS and MSS strains in the total number of staphylococcal strains isolated in healthcare settings, 2014-2019**

Setting	Isolated strains (total)		Strains tested to oxacillin* / cefoxitin **		including resistant (MRS)	
	abs	% (95% CI)	abs	% (95% CI)	abs	% (95% IC)
Inpatient department no.1	6510	55.2 (54.3-56.1)	5940	91.2 (90.6-92.0)	2375	40.0 (38.7-41.2)
Inpatient department no. 2	5293	44.8 (43.9-45.7)	4921	93.0 (92.2-93.6)	2022	41.0 (39.7-42.5)
Total	11 803	100.0	10 861	92.0 (91.6-92.5)	4397	40.5 (39.6-41.4)

*2014 – 2017 – methicillin-resistance determined by resistance to oxacillin
** 2018 – 2019 – methicillin-resistance determined by resistance to cefoxitin

In the multiannual dynamics, the issue of PSIs caused by MRS in healthcare settings is getting worse. The share of MRS strains isolated from patients with PSIs during 6 years of study increased, from 29.6% (95 CI 27.9-31.4) in 2014, to 55.9% (95 % CI 53.4-58.4) in 2019 (figure 2).

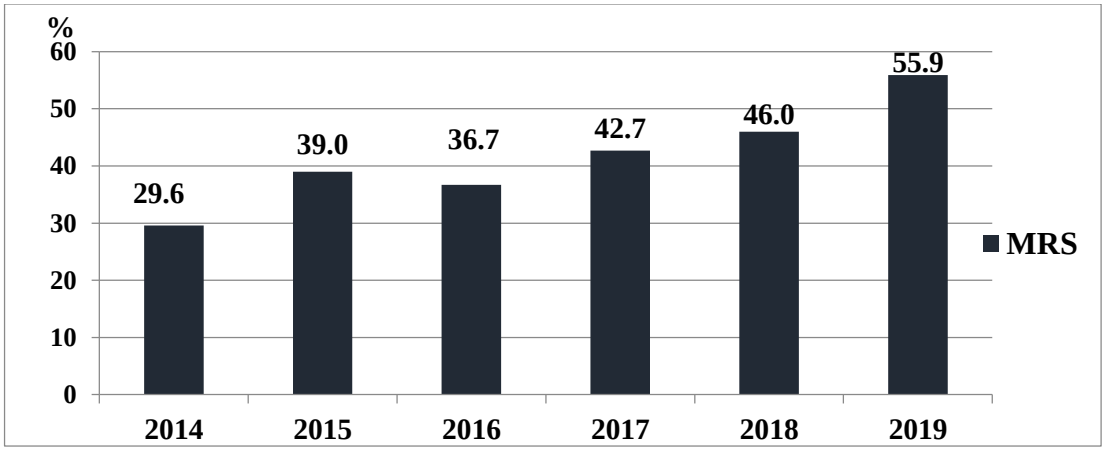


Figure 2. **Share of MRS strains in the total number of *Staphylococcus* strains in multiannual dynamics**

The study found that both coagulase-positive staphylococci and coagulase-negative staphylococci are likely to obtain resistance to methicillin.

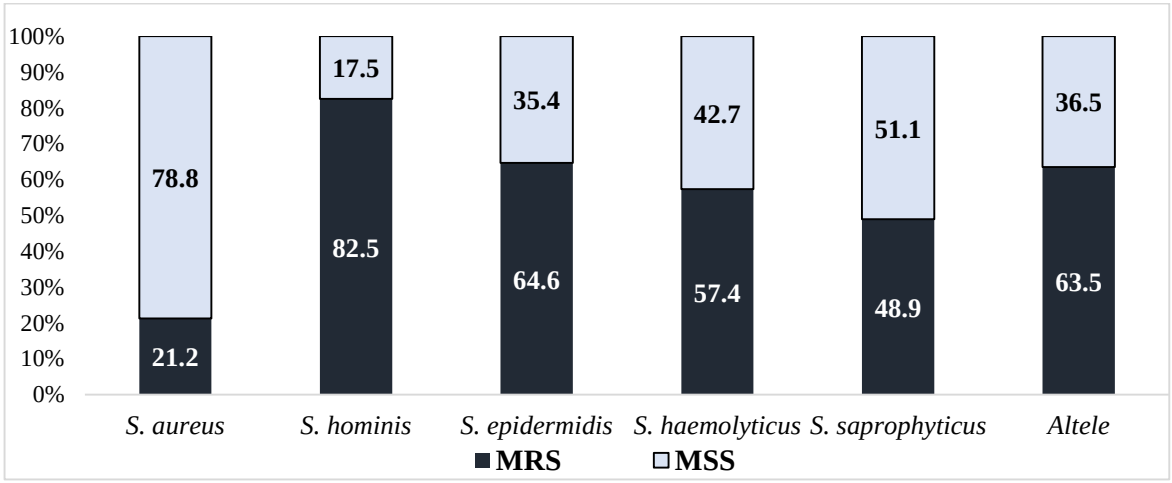


Figure 3. **Share of methicillin-resistant *Staphylococcus* strains in multiprofile hospitals, depending on staphylococcal species**

Of the total number of coagulase-positive *Staphylococcus* strains (*S. aureus*) tested for susceptibility / resistance to oxacillin / cefoxitin (n = 5410), resistance accounted for 21.2% (95% CI 20.2-22.4). A much higher degree of resistance to oxacillin / cefoxitin was found in coagulase-negative *Staphylococcus strains*. *S. hominis* strains showed resistance to oxacillin / cefoxitin in 82.5% (95% CI 75.5-88.8) cases, *S. epidermidis* - in 64.6% (95% CI 62.4-66.7), *S. haemolyticus* - in 57.4% (95% CI 55.5-59.2), *S. saprophyticus* - in 48.9% (95% CI 44.8-53.0). Other species of coagulase-negative staphylococci isolated in smaller shares (*S. capitis, S. sciuri, S. warneri, S. lugdunensis*) showed resistance in 63.5% (95% CI 53.1-73.1) (figure 3).

The level of MRS spread also varies depending on the department profile. More affected by MRS were: departments of traumatology and orthopedics 49.4% (95% CI 46.4-52.5) – inpatient department no. 2; department of surgery 32.2% (95% CI 29,7-34,9) – inpatient department no.1 and 34,9% (95% CI 32,1-37,9) – inpatient department no. 2; anesthesia and intensive care - 23.6% (95% CI 21.3-26.1) – inpatient department no. 1 and 12.6% (95% CI 10.7-14.8) – inpatient department no. 2. In both settings, there was a significantly high level of isolation of MRS strains from outpatients 21.1% (95% CI 19.1-23.2) – inpatient department no. 1 and 7.2% (95% CI 5,7-8,8) – inpatient department no. 2.

Depending on the pathological material from which they were isolated, staphylococcal strains showed a varying methicillin resistance. An obviously high share of MRS was found in blood cultures. Thus, of the total number of staphylococcal strains isolated from blood (n = 201), 153 strains were methicillin-resistant, accounting for 76.1% (95% CI 69.6-81.8). Also, staphylococcal strains isolated from the bile showed increased oxacillin resistance - 48.5% (95% CI 42.9-54.0). Staphylococcal strains isolated from urogenital tract samples showed oxacillin resistance in 22.9% (95% CI 19.4-26.8) and 16.7% (95% CI 14, 6-18,9) in respiratory samples.

The high share of MRS strains isolated from the blood is a serious issue, accounting for generalized infections (pneumonia, septicemia, etc.). Of the total number of patients with PSIs caused by methicillin-resistant *Staphylococcus,* 62.3% (56.9-67.9) had generalized infections, including pulmonary sepsis (25.3%, 95% CI 20,5-30.5), abdominal sepsis (21.5%, 95% CI 17.0-26.5), skin sepsis (7.4%, 95% CI 4.7-10.9), urosepsis (8.3%, 95% CI 5.5-12.0), and only 37.8% (95% CI 32.1-43.1) had localized forms, including inflammation / lung damage (9.0%, 95% CI 6.0-12.7), infected wounds (14.7%, 95% CI 11.0-19.2), spondyloarthritis (13.8%, 95% CI 10.2-18.1).

The MRS strains isolated in association with other species of microorganisms are of epidemiological and clinical significance. The analysis of the bacteriological results of the pathological materials, collected from patients with purulent-septic infections in multiprofile hospitals, found that MRS strains in 62.0% (95% CI 51.7-71.5) of cases were isolated in monocultures, and 38.0% (95% CI 28.6-48.4) of cases - in associations with other species of microorganisms. Associations with a single species of microorganisms constituted - 25.5% (95% CI 17.3-35.2), with two species of microorganisms - 10.9% (95% CI 5.5-18.7) and associations with 3 species of microorganisms were found in 1.7% (95% CI 0.2-6.6) of cases, which greatly complicates the course of diseases caused by MRS.

3.2. Spread of purulent-septic infections with methicillin-resistant *Staphylococcus* in maternity wards

The study revealed a high share of MRS strains in the maternity ward. Of the total number of staphylococcal strains isolated and tested for oxacillin susceptibility / resistance, 61.8% (95%

CI 56.1-67.3) of strains were oxacillin resistant (MRS), and only 38.2% (95% CI 32.7-43.9) strains were oxacillin susceptible (MSS).

Table 2. **Share of MRS strains in the total number of *Staphylococcus* strains isolated from patients with PSIs in maternity wards, depending on staphylococcal species**

Staphylococcal species	**Isolated strains (total)**	**Strains tested to oxacillin**		**including**			
				resistant (MRS)		**sensitive (MSS)**	
	abs	**abs**	**%**	**abs**	**% (95% CI)**	**abs**	**% (95% CI)**
S. aureus	124	124	100.0	54	43.5 (34.7-52.7)	70	56.5 (47.3-65.3)
S. epidermidis	202	185	91.6	137	74.1 (67.1-80.2)	48	25.9 (19.8-32.9)
Total	326	309	94.8	191	61.8 (56.1-67.3)	118	38.2 (32.7-43.9)

3.3. Spread of purulent-septic infections with methicillin-resistant *Staphylococcus* in rural healthcare settings

In terms of the spread of methicillin-resistant *Staphylococcus* strains in rural healthcare settings, it was found that the share of methicillin-resistant *Staphylococcus* strains averaged out to 22.4% (95% CI 19.5-25.4).

Table 3. **Share of MRS strains isolated from patients with PSIs in rural healthcare settings**

Characteristics of *Staphylococcus* strains		**indices**	**Administrative territory**			
			Criuleni	**Ialoveni**	**Strașeni**	**Total**
Isolated strains (total)		abs	316	490	284	1090
Strains tested to oxacillin		abs	204	382	201	787
		% (95% CI)	64.6 (59.0-69.8)	77.9 (74.0-81.6)	70.8 (65.1-76.0)	72.2 (69.4-74.8)
including	resistant (MRS)	abs	54	68	54	176
		% (95% CI)	26.5 (20.6-33.1)	17.8 (14.1-22.0)	26,9 (20.9-33.6)	22,4 (19.5-25.4)
	sensitive (MSS)	abs	150	314	147	611
		% (95% CI)	73.5 (66.1-79.4)	82.2 (78.0-85.9)	73.1 (66.4-79.1)	77.6 (74.6-80.5)

In Strașeni district, of the total number of *Staphylococcus* strains isolated from patients with PSIs, oxacillin-resistant staphylococcal strains constituted 26.9% (95% CI 20.9-33.6), in Criuleni district - 26.5% (95% CI 20.6-33.1) and 17.8% (95% CI 14.1-22.0) in Ialoveni district (table 3).

3.4. Assessment of the level of spread of methicillin-resistant *Staphylococcus* strains in the Republic of Moldova

Of the total of 13 219 *Staphylococcus* strains isolated from patients with purulent-septic infections both in multiprofile hospitals, maternity wards and healthcare settings in rural areas, the

average level of spread of methicillin-resistant *Staphylococcus* strains in the Republic of Moldova was determined, accounting for 39.8% (95% CI 38.9-40.7).

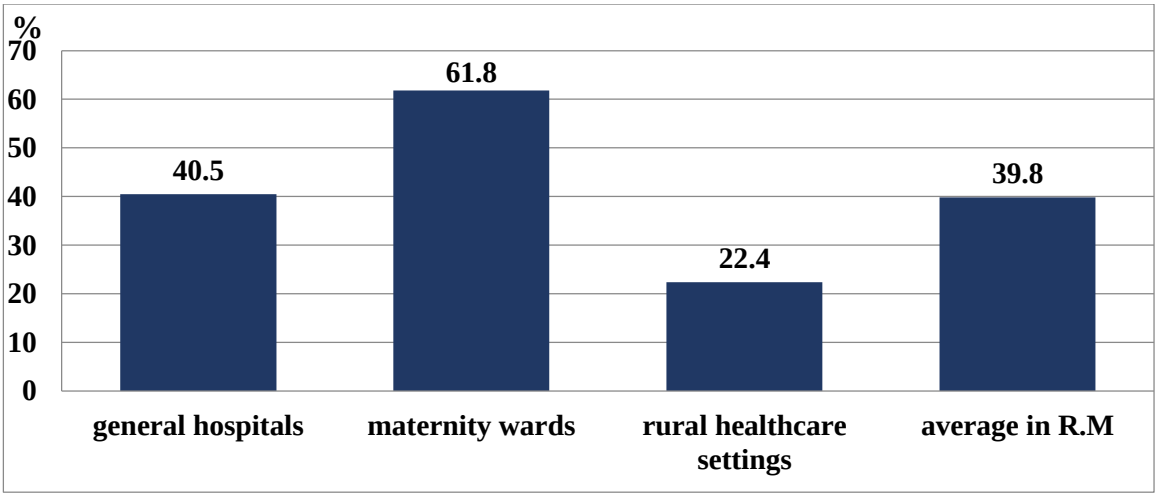


Figure 4. **The level of spread of methicillin-resistant *Staphylococcus* strains in the Republic of Moldova**

The epidemiological study found significant differences between the spread of methicillin-resistant *Staphylococcus* strains in multiprofile hospitals and the living environment. The highest frequency of MRS isolation was found in maternity wards 61.8% (95% CI 56.1-67.3), followed by multiprofile hospitals - 40.5% (95% CI (39.6-41.4). A lower frequency of 22.4% (95% CI 19.5-25.4) of MRS isolation was found in patients in rural healthcare settings (figure 4).

4. ANTIBIOTIC RESISTANCE/ MULTI-DRUG RESISTANCE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS* STRAINS

4.1. Antibiotic resistance of methicillin-resistant *Staphylococcus* strains

Analysis of antibioticograms of MRS strains isolated from patients with PSIs found a high share of antibiotic resistance. Antimicrobial resistance of methicillin-resistant *Staphylococcus* strains varied with both the group of antibiotics and the type of antibiotics. According to EUCAST (European Committee on Antimicrobial Susceptibility Testing), methicillin-resistant *Staphylococcus* strains (oxacillin / cefoxitin resistant) are resistant to beta-lactam antibiotics, a fact observed in the study.

The resistance of MRS strains to non-beta-lactam antibiotics has varied. The strains isolated from healthcare settings showed a higher degree of resistance to macrolides, fluoroquinolones, lincosamides, aminoglycosides, tetracyclines, phenicols, and higher susceptibility to glycopeptides, oxazolidinones, sulfamides (figure 5A).

Although the share of MRS strains in the maternity ward is quite high, the spectrum of antibiotics to which the susceptibility / resistance is tested is quite narrow, the strains also show a high degree of resistance to antibiotics tested (figure 5B).

The MRS strains isolated in rural healthcare settings showed a high resistance to macrolides, tetracyclines, sulfamides, aminoglycosides, phenicols, and an increased susceptibility to glycopeptides (figure 5C).

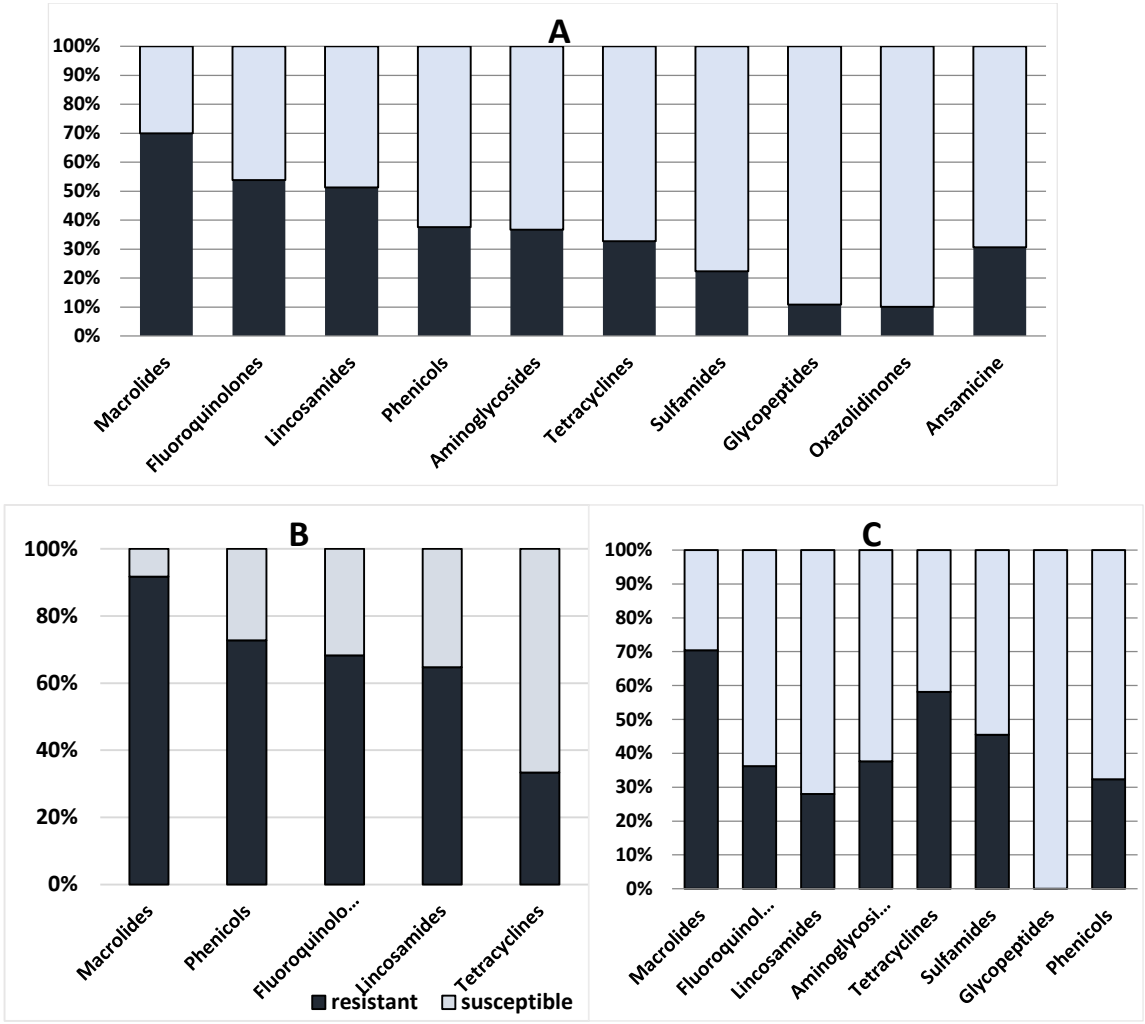
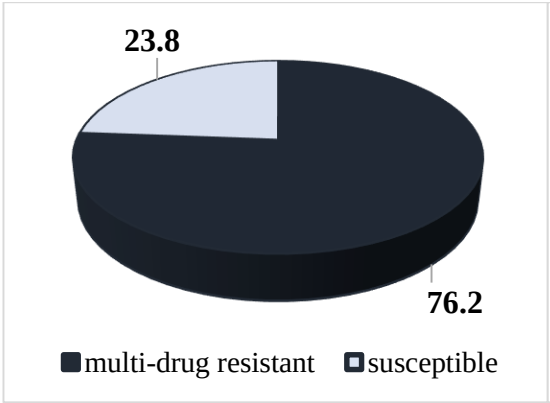


Figure 5. **Resistance to non-beta-lactam antibiotics of MRS strains isolated from patients with PSIs in multi-profile hospitals (A), maternity ward (B) and rural area (C)**

4.2. Multi-drug resistance of methicillin-resistant *Staphylococcus* strains

The MRS strains isolated from patients with PSIs were found to be multi-drug resistant to antibiotics - 76.2% (95% CI 74.6-77.8) (figure 6). The analysis of multi-drug resistance of MRS strains in the multiannual dynamics accounts for an alarming situation. Thus, multi-drug resistance of MRS strains increased from 79.0% (95% CI 75.8-81.9) in 2014 to 89.9% (95% CI 87.3-92.1) in 2017 (figure 7).



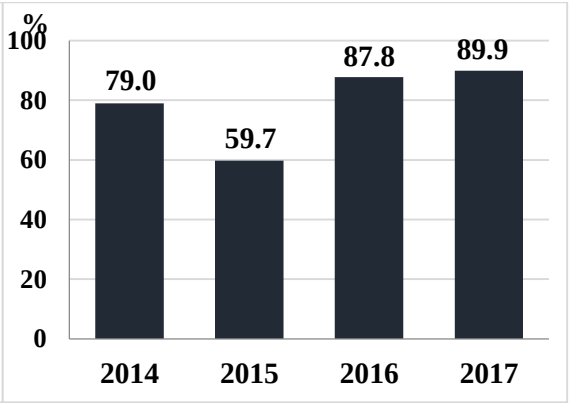


Figure 6. Multi-drug resistance of MRS strains

Figure 7. Multi-drug resistance of MRS strains in multiannual dynamics

4.3. Antibiotic resistance of *Staphylococcus* strains isolated from blood

The study of antibiograms of MRS and MSS blood cultures isolated from patients with sepsis found an increased antibacterial resistance of MRS strains, compared to MSS strains that showed, in most cases, high susceptibility to antibiotics (figure 8).

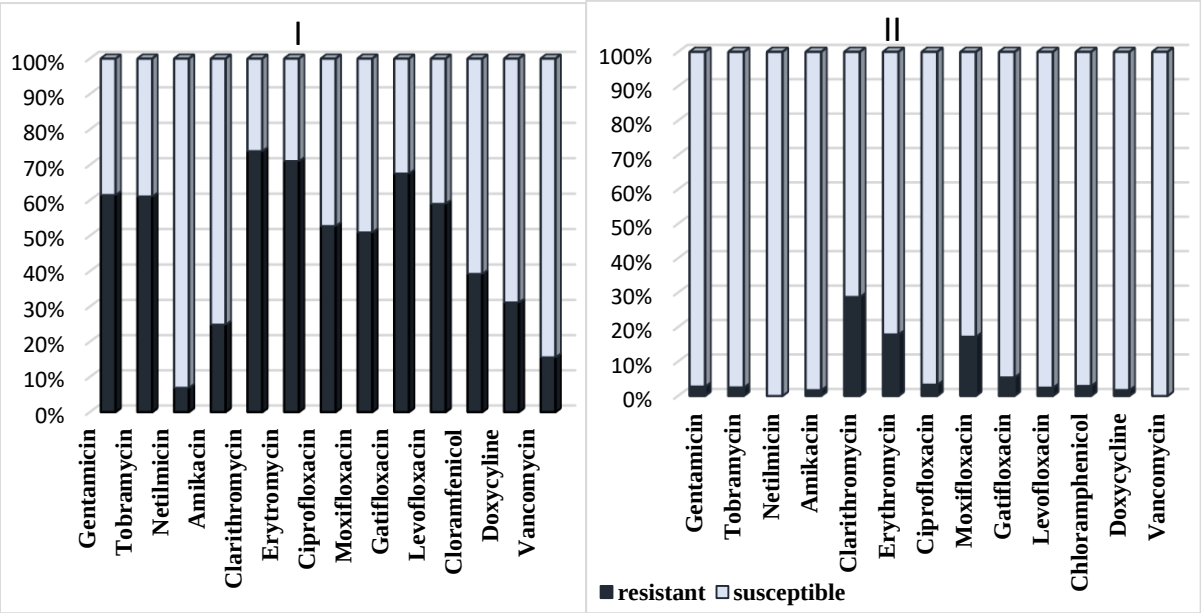


Figure 8. **Resistance / susceptibility to non-beta-lactam antibiotics of MRS (I) and MSS (II) strains isolated from patients with septicemia**

5. RISK FACTORS, ANTIBACTERIAL MANAGEMENT AND SOCIO-ECONOMIC IMPACT IN PSIs WITH METHICILLIN-RESISTANT *STAPHYLOCOCCUS*

5.1. Risk factors in PSIs with methicillin-resistant *Staphylococcus*

Table 4. **Comparative assessment of risk factors in MRS infections according to RR (OR)**

Risk factors	Septicemia RR (OR) (95% CI)	Trophic ulcers RR (OR) (95% CI)	Inflammations/ lung damage RR (OR) (95% CI)	Total RR (OR) (95% CI)	Degree of risk
Age ≥ 50 years	1.8 (1.1-3.2)	1.5 (0.4-5.2)	1.8 (0.8-4.1)	1.3 (1.0-1.9)	V
Patient`s gender (male)	1.2 (0.7-2.0)	1.1 (0.5- 2.2)	1.1 (0.5-2.6)	1.02 (0.7-1.5)	VI
Type of emergent hospitalization	2.1 (1.2-3.7)	1.2 (0.5-3.1)	1.2 (0.5-2.7)	1.4 (1.0-2.1)	IV
Frequency of hospitalizations	1.9 (1.1-3.2)	5.2 (2.3-12.1)	3.5 (1.5-8.2)	3.2 (2.2- 4.7)	III
Presence of surgical interventions	2.3 (1.3-4.0)	1.1 (0.2-6.7)	2.0 (0.4-10.0)	1.4 (1.0-2.1)	IV
Microbial associations	2.4 (1.4- 4.2)	16.5 (6.1-44.9)	6.1 (2.1-17.8)	4.8 (3.2-7.3)	I
Exposure to treatment in ICU	4.0 (2.1-7.3)	5.2 (1.6-17.1)	4.3 (1.7-10.9)	4.5 (3.0-6.7)	II

The comparative assessment of risk factors in MRS infections found that the risk factors which greatly complicate the clinic and treatment of patients, were as follows: microbial associations RR (OR) - 4.8 (95% CI 3.2 – 7.3), treatment exposure in ICU RR (OR) - 4.5 (95% CI 3.0 - 6.7), repeated hospitalizations RR (OR) - 3.2 (95% CI 2.2 - 4.7), the presence of surgeries RR (OR) - 1.4 (95% CI 1.0 - 2.1), emergent hospitalizations RR (OR) - 1.4 (95% CI 1.0 - 2.1), and people over the age of 50 RR (OR) - 1.3 (95% CI 1.0 - 1.9) (table 4).

5.2. Antibacterial therapeutic management of patients with methicillin-resistant *Staphylococcus* infections

Based on table 5, which summarizes the antibacterial therapeutic management of patients with MRS and MSS, it was found that patients with PSIs with MRS were administered from 1 to 9 antimicrobial preparations as part of antimicrobial therapy. After comparing the resistance /susceptibility of MRS strains in patients receiving antibiotic therapy, using antibioticograms, it was found that in 53.2% (95% CI 49.7-56.7) MRS strains proved to be resistant to administered antibiotics, 24.7% (95% CI 21.7-27.8) of cases were not tested for susceptibility / resistance, and only in 22.1% (95% CI 19.3-25.2) of cases isolated strains showed susceptibility to administered antibiotics. On average, only one patient received 3 antimicrobial preparations.

Table 5. **Number of antibiotics administered to a patient with MRS in monocultures and microbial associations, including antimicrobial resistance / susceptibility of MRS strains**

Number of administered antibiotics			**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Total**
Research group (MRS)													
Number of patients	abs		5	54	58	63	41	28	14	1	4	1	269
	%		1.9	20.1	21.6	23.4	15.2	10.4	5.2	0.4	1.5	0.4	100.0
Total administered antibiotics			0	54	116	189	164	140	84	7	32	9	795
including MRS susceptibility to administered antibiotics	R	abs	-	32	63	103	90	75	45	3	11	1	423
		%	-	59.3	54.3	54.5	54.9	53.6	53.6	42.9	34.4	11.1	53.2
	S	abs	-	6	25	40	38	34	15	2	13	3	176
		%	-	11.1	21.6	21.2	23.2	24.3	17.9	28.6	40.6	33.3	22.1
	not tested	abs	-	16	28	46	36	31	24	2	8	5	196
		%	-	29.6	24.1	24.3	22.0	22.1	28.6	28.6	25.0	55.6	24.7
Control group (MSS)													
Number of patients	abs		7	102	54	18	7	3	2	-	-	-	193
	%		3.6	52.8	28.0	9.3	3.6	1.6	1.0	-	-	-	100.0
Total antibiotics adm.			-	102	108	54	28	15	12	-	-	-	319
including MSS susceptibility to administered antibiotics	R	abs	-	1	1	-	1	-	-	-	-	-	3
		%	-	1.0	0.9	-	3.6	-	-	-	-	-	0.9
	S	abs	-	81	86	46	18	13	11	-	-	-	255
		%	-	79.4	79.6	85.2	64.3	86.7	91.7	-	-	-	79.9
	not tested	abs	-	20	21	8	9	2	1	-	-	-	61
		%	-	19.6	19.4	14.8	32.1	13.3	8.3	-	-	-	19.1

In terms of the therapeutic management of patients with MSS infections, it proved to be much more effective, as MSS strains showed resistance to antimicrobial preparations administered only in 0.9% (95% CI 0.2-2.7) and, conversely, susceptibility in 79.9% (95% CI 75.1-84.2). Patients with MSS were administered a smaller number of antibiotics, compared to those with MRS, from 1 to 6 antibiotics. On average, only one patient received 1.7 antimicrobial preparations (table 5).

5.3. Socio-economic impact of infections caused by methicillin-resistant *Staphylococcus* strains

Based on table 6, which summarizes the impact of patients compared to the number of MRS and MSS infections, according to the main criteria (number of hospital bed days, expenses per patient, lethality rate), the situation is much more serious in patients with MRS, differences between hospital bed days (19.5 days vs 12.8 days) and expenses (11 272 lei vs 7530 lei per patient), the lethality rate (20.4% vs 4.1%) being significant in the groups of patients with MRS and MSS.

Table 6. **Mean comparative impact in MRS and MSS infections**

Nosological form	**Number of bed days**		**Expenses per patient (lei)**		**Lethality rate (%)**	
	MRS	MSS	MRS	MSS	MRS	MSS
Septicemia	21.1	15.7	12 660	9 438	28.9	7.5
Inflammation/lung damage	18.3	11.3	11 010	6 780	8.6	2.5
Trophic ulcers	16.9	10.6	10 146	6 372	7.7	1.4
M_{as} (lots/groups of patients)	19.5	12.8	11 272	7 530	20.4	4.1

6. DISCUSSIONS

Antimicrobial resistance is a global priority. According to the WHO, antimicrobial resistance has reached alarming levels in many regions of the world [14, 15]. The development of antibiotic resistance, including *Staphylococcus* strains, is one of the great challenges facing humanity today. Since the first reporting of methicillin resistance in *Staphylococcus* strains in 1961, to date, the scale of the problem has increased dramatically, and monitoring of variations in antibiotic sensitivity of *Staphylococcus* in different countries has become a mandatory concern.

Studies found considerable variations in the level of spread of MRSA strains in European countries. A north-south gradient is observed, MRSA strains being rare in Scandinavian hospitals (< 2%) and much more widespread in hospitals in Mediterranean countries (> 40%) [7, 8, 9].

In most EU / EEA countries, as a result of implemented intervention programs to limit the incidence of severe MRSA infections, in recent years the percentage of methicillin-resistant *Staphylococcus aureus* strains decreased from 19.6% in 2014 to 16.9% in 2017 [12, 13].

The study showed that methicillin-resistant *Staphylococcus* infections are a major public health problem in the Republic of Moldova. The level of spread of methicillin-resistant staphylococcal strains in the Republic of Moldova is high, averaging 39.8% (95% CI 38.9-40.7), and varies depending on the type of healthcare setting. The highest share is found in maternity wards 61.8% (95% CI 56.1-67.3), followed by healthcare settings 40.2% (95% CI (39.6-41.4), a lower level of MRS spread being found in rural healthcare settings - 22.4% (95% CI 19.5-25.4).

In the multiannual dynamics, the share of methicillin-resistant *Staphylococcus* strains increased from 29.6% (95% CI 27.9-31.4) in 2014 to 55.9% (95% CI 53.4-58.4) in 2019, reaching high levels in blood cultures (76.1% (95% CI 69.6-81.8), as well as for coagulase-negative staphylococci (59.6% (95% CI 58.3-60.9). There is currently an alarming situation in the Republic of Moldova in terms of maintaining antimicrobial resistance, which imposes the need for emergency measures at all levels. Knowledge of local epidemiology in purulent-septic nosocomial infections with MRS will contribute to the implementation and provision of rational measures for surveillance and control of this issue.

Methicillin resistance of *Staphylococcus* strains has a negative impact on clinical and economic outcomes. MRS infections significantly increase economic costs by prolonging hospitalization, enhancing incapacity for work, augmenting the use of antibiotics and other treatment methods, and increasing the need for additional diagnostic tests [20, 21, 22, 23, 24, 25].

The results obtained in the study show that in patients with MRS infections the duration of hospitalization is much longer compared to that of patients with MSS infections, and the antibacterial therapeutic management of MRS patients proved to be much more complicated, compared to patients with MSS, the latter requiring a complex treatment over a longer period, as well as a larger number of hospital stays, including in intensive care units. Also, the lethality rate of patients with purulent-septic infections caused by MRS is much higher compared to that of patients with MSS, reaching on average 20.4%, compared to 4.1%, which demonstrates both the severity and the relevance of the problem, as well as the need for implementing rational surveillance and control measures.

THESIS CONCLUSIONS

1. Nosocomial infections with MRS are currently a major public health problem. The level of spread of methicillin-resistant *Staphylococcus* strains in the Republic of Moldova constitutes on average 39.8% (95% CI 38.9-40.7) of the total *Staphylococcus* strains and varies depending on the type of healthcare setting. The highest share is found in maternity wards 61.8% (95% CI 56.1-67.3), followed by healthcare settings 40.2% (95% CI (39.6-41.4), a lower level of spread of MRS is recorded in rural healthcare settings 22.4% (95% CI 19.5-25.4). In the multiannual dynamics the share of MRS strains increased from 29.6% (95% % CI 27.9-31.4) in 2014 to 55.9% (95% CI 53.4-58.4) in 2019.

2. The severity of the clinical forms is a peculiarity of nosocomial purulent-septic infections caused by MRS, the generalized forms being predominant 62.3% (56.9-67.9). The high rate of 76.1% (95% CI 69.6-81.8) of methicillin-resistant *Staphylococcus* (MRS) strains in blood cultures confirms this fact. This phenomenon is largely favored by microbial associations - 38.0% (95% CI 28.6-48.4), thus greatly complicating both the clinical course and treatment of these infections.

3. Although coagulase-positive staphylococcus is more common in human purulent-septic infections, the degree of methicillin resistance was found to be lower - 21.2% (95% CI 20.2-22.4), compared to coagulase-negative staphylococci - 59.6% (95% CI 58.3-60.9).

4. The most affected departments by PSIs with MRS in multiprofile PHCS proved to be the following departments: traumatology and orthopedics, surgery, anesthesia and intensive care. At the same time, a large part of the MRS strains in both settings were isolated from patients in the consulting rooms as well as from patients receiving outpatient treatment, which demonstrates that it is likely to import MRS strains in hospitals.

5. It was found that MRS strains also show a much higher resistance to all groups of antibiotics compared to MSS strains. The investigations confirm a high resistance of MRS to beta-lactamases, and compared to non-beta-lactam antibiotics, the resistance of MRS strains varies depending on the group of antibiotics. 76.2% (95% CI 74.6-77.8) of the MRS strains proved to be multi-drug resistant to antibiotics. This index increased in the multiannual dynamics from 79.0% (95% CI 75.8-81.9) in 2014 to 89.9% (95% CI 87.3-92.1) in 2017.

6. Microbial associations, treatment in intensive care units, urgent and repeated hospitalizations, the presence of surgery and people over 50 years of age have been shown to be risk factors for MRS infections.

7. Irational antibacterial management in the treatment of patients with MRS is an important phenomenon. Patients with MRS strains isolated in monocultures and microbial associations were indicated from 1 to 9 antibiotics, to which only 22.1% (95% CI 19.3-25.2) of the strains showed susceptibility. Although the resistance of MRS strains to cephalosporins is high, they continue to be used in the treatment of MRS infections.

8. The socio-economic impact of patients with PSIs with MRS is much higher compared to patients with MSS, the latter requiring a more complex treatment over a longer period and a larger number of days of hospital stay. The consequences of MRS infections are much more serious, the lethality being about 5 times higher, compared to MSS infections, which accounts for both the severity and relevance of the problem.

RECOMMENDATIONS

1. Based on the current issue of accelerated spread and increased antibiotic resistance of methicillin-resistant *Staphylococcus*, it is necessary to intensify the surveillance of methicillin-resistant *Staphylococcus* infections which should include assessment of the level of MRS spread, detection, recording, monitoring and analysis of cases of MRS infections, systematic evaluation of antibiotic resistance of MRS strains, determination of risk factors and departments/units exposed to risk in all healthcare settings, implementation of strict surveillance and control measures, including the rational use of antibiotics.

2. Upon the admission to the hospital, the patients transferred from other inpatient departments, as well as the patients with indications for complicated surgeries in case of planned hospitalizations require compulsorily to be subjected to the bacteriological investigation for MRS detection.

3. It is practically impossible to effectively treat patients with NPSIs, especially caused by MRS, without any microbiological diagnosis which entails the use of an antibiogram. Hence, it is necessary to make the microbiological diagnosis more efficient as the main means in the surveillance and control of MRS infections by equipping microbiological laboratories with specialists, modern technology and other means necessary to ensure timely and qualitative microbiological diagnosis. The determination of MRS strains urgently requires an extended antibiogram to a wider spectrum of antibiotics, including those in the hospital reserve.

4.The increasing resistance of MRS strains to several antimicrobial agents necessitates the prudent use of antibacterial preparations, in accordance with the individual results of antimicrobial susceptibility testing for isolated strains of each patient.

5. In order to reduce the risk of dissemination and contamination of other patients, it is necessary to implement strict control measures, namely, to isolate patients with MRS in separate wards, specially designed for such patients, and to detect MRS carriers among patients upon admission as well as among medical workers, and to subject them to treatment.

6. Supplementing antibiotics as needed by healthcare settings, based on the results of studies on susceptibility / resistance of circulating strains in the setting, is mandatory. The rational use of antibiotics will make the treatment efficient and will reduce antibiotic resistance.

REFERENCES

1. Prisacari V. *Ghid de supraveghere şi control în infecţiile nosocomiale.* Chişinău, 2009; ed. II: 9-10; 27; 182.
2. Prisacari V, Berdeu I. Problema antibioticorezistenței microbiene. *Akademos.* 2014; 1(32): 92-100.
3. European Centre for Disease Prevention and Control. Point prevalence survey of healthcareassociated infections and antimicrobial use in European acute care hospitals – protocol version 5.3. Stockholm: ECDC; 2016. Disponibil la: https://www.ecdc.europa.eu/sites/default/files/documents/PPS-HAI-AMR-protocol.pdf [accesat la 5.12.2019].
4. Friedrich AW. Control of hospital acquired infections and antimicrobial resistance in Europe: the way to go. *Wien Med Wochenschr*. 2019; 169(1): 25-30.
5. Busuioc E, Cateriniuc N, Furtuna N, Rata V, Surdu Ş. Pilotarea studiului de prevalență de moment a infecțiilor asociate asistenței medicale și consumul antimicrobienelor în spitalele din Republica Moldova. *Sănătate Publică, Economie şi Management în Medicină*. 2019; 4(82): 299-303.
6. Turner NA, Sharma-Kuinkel BK, Maskarinec SA, Eichenberger EM, Shah PP, Carugati M, et al. Methicillin-resistant *Staphylococcus aureus:* an overview of basic and clinical research. *Nat Rev Microbiol.* 2019; 17(4): 203-218.
7. Johnson AP. Methicillin-resistant *Staphylococcus aureus*: the European landscape. *J Antimicrob Chemother*. 2011; 66 Suppl 4: iv43-iv48.
8. Fluit AC, Wielders CL, Verhoef J, Schmitz FJ. Epidemiology and susceptibility of 3,051 Staphylococcus aureus isolates from 25 university hospitals participating in the European SENTRY study. *J Clin Microbiol.* 2001; 39(10): 3727-32.
9. Stenhem M, Ortqvist A, Ringberg H, Larsson L, Olsson-Liljequist B, Haeggman S et al. Epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) in Sweden 2000-2003, increasing incidence and regional differences. *BMC Infectious Diseases*. 2006; 6:30.
10. Friedrich AW. Control of hospital acquired infections and antimicrobial resistance in Europe: the way to go. *Wien Med Wochenschr*. 2019; 169(1): 25-30.
11. Lakhundi S, Zhang K. Methicillin-Resistant *Staphylococcus aureus:* Molecular Characterization, Evolution, and Epidemiology. *Clin Microbiol Rev.* 2018; 31(4): e00020-18.
12. European Centre for Disease Prevention and Control. Surveillance of antimicrobial resistance in Europe 2016. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Stockholm:ECDC; 2017. Disponibil la: https://www.ecdc.europa.eu/en/publications-data/surveillance-antimicrobial-resistance-europe-2017 [accesat 12 martie 2020].
13. European Centre for Disease Prevention and Control. Surveillance of antimicrobial resistance in Europe. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net) 2017. Stockholm:ECDC; 2018. Disponibil la: https://www.ecdc.europa.eu/en/publications-data/surveillance-antimicrobial-resistance-europe-2018 [accesat 12 ianuarie 2020].

14. Global priority list of antibiotic - rezistant bacteria to guide research, discovery and development of new antibiotics. Geneva, World Health Organization, 2017. Disponibil la: https://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/ [accesat la 10.06.2020].

15. Antimicrobial resistance: global report on surveillance 2014. Geneva, World Health Organization. Disponibil la: https://www.who.int/drugresistance/documents/surveillancereport/en/ [accesat 24 martie 2019].

16. Lee JYH, Monk IR, Gonçalves da Silva A, Seemann T, Chua KYL, Kearns A, et al. Global spread of three multidrug-resistant lineages of *Staphylococcus epidermidis. Nat Microbiol.* 2018; 3(10): 1175-1185.

17. Sahal G, Bilkay IS. Multi drug resistance in strong biofilm forming clinical isolates of *Staphylococcus epidermidis. Braz J Microbiol.* 2014; 45(2): 539-544.

18. Becker K, Heilmann C, Peters G. Coagulase-negative staphylococci. *Clin Microbiol Rev.* 2014; 27(4):870-926.

19. Longauerova A. Coagulase negative staphylococci and their participation in pathogenesis of human infections. *Bratisl Lek Listy.* 2006; 107(11-12): 448–452.

20. Wernitz MH, Keck S, Swidsinski S, Schulz2 S, Veit1 K. Cost analysis of a hospital-wide selective screening programme for MRSA carriers in the context of diagnostic related groups (DRG) payment. *Clin Microbiol Infect.* 2005; 11: 466-471

21. Antonanzas F, Lozano C, Torres C. Economic features of antibiotic resistance: the case of methicillin-resistant *Staphylococcus aureus. Pharmacoeconomics.* 2015; 33: 285–325.

22. Joo EJ, Park DA, Kang CI, Chung DR, Song JH, Lee SM, et al. Reevaluation of the impact of methicillin-resistance on outcomes in patients with *Staphylococcus aureus* bacteremia and endocarditis. *Korean J Intern Med.* 2019; 34(6): 1347-1362.

23. Prisacari V, **Buga D**, Berdeu I. Aspecte epidemiologice în ulcerele trofice cu *Staphylococcus* meticilin-rezistent. *One Health & Risk Management.* 2021; 2: 51-57.

24. Anderson DJ, Kaye KS, Chen LF, et al. Clinical and financial outcomes due to methicillin resistant Staphylococcus aureus surgical site infection: a multi-center matched outcomes study. *PLoS One.* 2009; 4(12): e8305.

25. Thampi N, Showler A, Burry L, Bai AD, Steinberg M, Ricciuto DR, et al. Multicenter study of health care cost of patients admitted to hospital with *Staphylococcus aureus* bacteremia: Impact of length of stay and intensity of care. *Am J Infect Control.* 2015; 43(7):739-44.

26. Diaz R, Afreixo V, Ramalheira E, Rodrigues C, Gago B. Evaluation of vancomycin MIC creep in methicillin-resistant *Staphylococcus aureus* infections-a systematic review and meta-analysis. *Clin Microbiol Infect.* 2018; 24(2): 97-104.

27. Almaş A, Flonta M, Petraşcu M, Năstase V. Sensibilitatea la antibiotice a tulpinilor de *Staphylococcus aureus* izolate din infecţii ale tegumentelor şi părţilor moi. *Clujul Medical.* 2011; 84(2): 173-7.

28. Prisacari V, **Buga D**, Berdeu I. Nosocomial infections with methicillin resistant *Staphylococcus:* epidemiogenic situation at day, solutions. *AKADEMOS.* 2017; 4(47): 72-76.

29. Balan G, Burduniuc O, Siniţîna I, Iasâbaş O, Bunescu I. Frecvenţa izolării tulpinilor de *Staphylococcus aureus* meticilino-rezistente în Republica Moldova. *Buletin of the Academy of Sciences of Moldova. Medical Sciences.* 2017; 1(53): 22-23.

30. Balan G., Covantev S., Cazacu-Stratu A. et al. Frequency of methicillin-resistant *Staphylococcus aureus* strains in healthcare associated infections in the Republic of Moldova. *Romanian Archives of Microbiology and Immunology*. 2017; 79-84.

31. Galeţchi P, Buiuc D, Plugaru Ş. *Ghid de microbiologie medicală.* 1997. pp. 44:81, 165:170.

32. BioMerieux – diagnostics. Vitek 2 Compact. Disponibil pe: https://www.biomerieux-diagnostics.com/vitekr-2-compact-0 [accesat: 19.06.2021].

33. Buiuc D, Neguţ M. *Tratat de microbilogie clinică*. Ed. III, Editura medicală Bucureşti, 2009, pp. 562-582.

34. Prisacari V. *Epidemiologie generală. Bazele medicinei prin dovezi*. Chișinău. 2020; 181-187.

SCIENTIFIC PAPERS

- **Articles in international scientific journals abroad:**
  - ✓ **articles in ISI, SCOPUS and other international databases ***

1. **Буга Д.,** Присакарь В. Значимость метициллин-резистентных стафилококков в септической патологии. В: *Медицинский альманах*. 2019; 1: 40-43. ISSN: 1997-7689. doi: 10.21145/2499-9954-2019-1-40-43 (**IF:0,47**).
2. Prisacari V., **Buga D**. Epidemiology of purulent-septic infections caused by methicillin-resistant *Staphylococcus* in the Republic of Moldova. In: *The Medical-Surgical Journal* (în tipar). E-ISSN: 2286-2560 (**IF: 1.044**).
3. Присакарь В., **Буга Д**., Сава В. Внутрибольничные инфекции вызванные метициллинрезистентными стафилакокками (MRS). В: *Журнал МедиАль.* 2018; 2: 8-11 ISSN 2225-0026 (**IF: 0,356**).
4. Присакарь В., **Буга Д.** Проблема роста антибиотикорезистентности микроорганизмов рода *Staphylococcus.* В: *Журнал МедиАль.* 2020;(2):55. ISSN 2225-0026 (**IF: 0,356).**

- **Articles in accredited national scientific journals:**
  - ✓ **articles in category B journals**

5. Prisacari V., **Buga D**., Berdeu I. Infecțiile nosocomiale cu *Staphylococcus* meticilino-rezistent: situația epidemiogenă la zi, soluții. În: *AKADEMOS*. 2017; 4: 72-77. ISSN 1857-0461
6. **Buga D**., Prisacari V., Berdeu I., Balica I., Siniţîna I., Manica L., Rusu V. Situația epidemiogenă prin infecții cu *Staphylococcus* meticilin-rezistent în Republica Moldova. În: *Sănătate publică, Economie şi Management în Medicină*. 2019; 4 (82): 294-299. ISSN 1857-0461
7. **Buga D**. Problema creșterii rezistenței la preparatele antibacteriene a tulpinilor de *Staphylococcus.* În: *Sănătate publică, Economie şi Management în Medicină.* 2020; 5 (87): 26-31. ISSN 2587-3873
8. **Buga D**. Infecțiile septico-purulente cu *Staphylococcus* meticilin-rezistent: articol de sinteză. In: *Moldovan Journal of Health Sciences (Revista de Ştiinţe ale Sănătăţii din Moldova).* (în tipar).

- **Articles in journals under the process of accreditation:**

9. Prisacari V., **Buga D**., Berdeu I. Aspecte epidemiologice în ulcerele trofice cu *Staphylococcus* meticilin-rezistent. In: *One Health & Risk Management*. 2021; 2: 51-57. (**IF:0,01**).

● **Abstracts in the proceedings of national and international scientific conferences**

10. **Buga D.** Aspecte epidemiologice a infecțiilor nosocomiale septice cauzate de *Staphylococcus* meticilino-rezistent (MRS). În: *Volum de rezumate a manifestărilor ştiinţifice, zilele U.M.F din Craiova a XLIX-a ediţie*, Editura Universitară Craiova; 2019, p. 75.
11. **Buga D.** Incidența prin infecții nosocomiale cu *Staphylococcus* meticilin-rezistent în Republica Moldova. În: *Culegere de rezumate ştiinţifice ale studenţilor, rezidenţilor şi tinerilor cercetători, USMF* „Nicolae Testemiţanu". Chișinău; 2019, p. 36.
12. **Буга Д.** Чувствительность к антибиотикам штаммов *Staphylococcus* метициллин-устойчивых в родовспомогательных стационарах. *В: Мікробіологія, вірусологія та імунологія в сучасній клінічній і лабораторній медицині, Харків*; 2020, с.15.
13. **Buga D.** Epidemiogenic situation by infection with methicillin-resistant *Staphylococcus* in Republic of Moldova. In: *Abstract book, MedEspera 2020: The 8th International Medical Congress for Students and Young Doctors, Chişinău;* 2020. p. 305.
14. **Buga D.** Problema creșterii rezistenței la preparatele antibacteriene a tulpinilor de *Staphylococcus*. În: *Culegere de rezumate ştiinţifice ale studenţilor, rezidenţilor şi tinerilor cercetători.* USMF „Nicolae Testemiţanu; Chișinău; 2020, p. 169.
15. **Buga D.** Prisacari V. Infecțiile cu *Staphylococcus* meticilino-rezistent în spitalele multiprofil, în dinamica multianuală n: *Culegere de rezumate ştiinţifice ale studenţilor, rezidenţilor şi tinerilor cercetători.* USMF „Nicolae Testemiţanu; Chișinău; 2021.

● **Participation with papers in scientific forums:**

✓ **International forums**

16. **Буга Д. В.,** Присакарь В. И. Роль метициллин-резистентных стафилококков в септической патологии. *Всероссийская научно-практическая конференция специалистов по контролю ИСМП „Инфекции, связанные с оказанием медицинской помощи — междисциплинарный подход к профилактике" с международным участием.* Екатеринбург, 24-25 апреля 2019.
17. **Buga D.** Aspecte epidemiologice a infecțiilor nosocomiale septice cauzate de *Staphylococcus* meticilino-rezistent (MRS)". *Conferința ştiinţifică anuală în cadrul Zilelor U.M.F din Craiova a XLIX-a ediţie.* Craiova 7-8 iunie, 2019.
18. В.И. Присакарь, Н.И. Андронаки, **Д.В. Буга.** Проблема распространения и роста антибиотикорезистентности микроорганизмов рода *Acinetobacter* и *Staphylococcus. Ежегодная всероссийская научно-практическая конференция с международным участием. Актуальные проблемы эпидемиологии инфекционных и неинфекционных болезней 2020.* Москва, 21-23 октября 2020.

✓ **National forums with international participation**

19. Prisacari V., **Buga D.,** Berdeu I. Situaţia prin infecţii nosocomiale cu *Staphylococcus* meticilino-rezistent. *Conferinţa ştiinţifică „Optimizarea supravegherii epidemiologice în infecţiile nosocomiale".* Chișinău 13-14 octombrie, 2017.
20. **Buga D.** Epidemiologia Infecțiilor nosocomiale cu *Staphylococcus* meticilin rezistent în Republica Moldova. *Congresul al VIII-lea al specialiştilor din domeniul sănătăţii publice şi managementului sanitar.* Chișinău, 24-25 octombrie 2019.
21. Prisacari V., Baranețchi I., Spătaru D., **Buga D**., Andronache N., Sava V. Problema infecțiilor asociate asistenței medicale la zi. *Congresul al VIII-lea al specialiştilor din domeniul sănătăţii publice şi managementului sanitar.* Chișinău, 24-25 octombrie 2019.
22. **Buga D.,** Andronachi N. Problema răspândirii și creșterii rezistenței la antibiotice a tulpinilor de *Acinetobacter* și *Staphylococcus* meticilin-rezistent**.** *Noaptea cercetătorilor*

europeni 2020. Chișinău, 27 noiembrie 2020.

23. Prisacari V., **Buga D.,** Berdeu I. Infecţiile nosocomiale cu *Staphylococcus* meticilino-rezistent în Republica Moldova. *Conferința științifică „Maladiile infecțioase în lumea modernă: provocări și perspective”.* Chișinău, 26 martie 2021.

✓ **National**

24. **Buga D.** Incidenţa prin infecţii nosocomiale cu *Staphylococcus* meticilino-rezistent. *Conferința științifică anuală în cadrul Zilelor* USMF *„Nicolae Testemițanu”.* Chișinău, 15-19 octombrie 2018.
25. **Buga D.** Situația epidemiogenă prin infecții cu *Staphylococcus* meticilino-rezistent în Republica Moldova. *Conferința științifică anuală în cadrul Zilelor* USMF*„Nicolae Testemițanu”.* Chișinău, 15-18 octombrie 2019.
26. **Buga D.** Problema creșterii rezistenței la preparatele antibacteriene a tulpinilor de *Staphylococcus*. *Congresul Consacrat Aniversării a 75-a de la fondarea USMF „Nicolae Testemițanu”.* Chișinău, 20-23 octombrie 2020.
27. Prisacari V., **Buga D.,** Andronachi N. Problema răspândirii și antibioticorezistenței infecțiilor nosocomiale cu *Acinetobacter* și *Staphylococcus* meticilino-rezistent. *Seminarul științifico-practic în cadrul IMSP Spitalului Clinic Republican „Timofei Moșneaga”.* Chișinău, 03 februarie 2021.
28. Prisacari V., **Buga D.,** Andronachi N. Problema răspândirii și antibioticorezistenței infecțiilor nosocomiale cu *Acinetobacter* și *Staphylococcus* meticilino-rezistent. *Seminarul științifico-practic în cadrul IMSP Institutul de Medicină Urgentă.* Chișinău, 19 februarie 2021.
29. **Buga D.** Infecțiile cu Staphylococcus meticilino-rezistent în spitalele multiprofil, în dinamica multianuală. *Conferința științifică anuală în cadrul Zilelor USMF „Nicolae Testemițanu”.* Chișinău, 22-20 octombrie 2021.

● **Participation with posters in scientific forums:**

✓ **International**

30. **Buga D**., Prisacari V. Situația epidemiologică a infecțiilor cu *Staphylococcus* meticilin-rezistent în Republica Moldova. A XII-a Conferință Națională de Microbiologie și Epidemiologie: *„Microbiologia şi Epidemiologia Românească – Realizări, Evoluţii şi Perspective”,* București, 14-16 noiembrie 2019.

✓ **National with international participation**

31. **Buga D**. Epidemiogenic situation by infection with methicillin-resistant *Staphylococcus* in Republic of Moldova. MedEspera 2020: *The 8th International Medical Congress for Students and Young Doctors*, Chisinau, 24-26 september 2020.