Original article

Impact of the COVID-19 pandemic on the antimicrobial resistance at the General Hospital level in Republic of Srpska, Bosnia and Herzegovina

**Nasta Manojlović[1], Biljana Mijović[1,2,6],
Zlatko Maksimović[1], Dragana Sokolović[3],
Sanda Lazić[1], Ljubica Bojanić[4],
Dragana Drakul[3], Nenad Lalović[5,6],
Marija Lukić[7], Bojan Joksimović[8]**

[1]PHI Hospital "Sveti vračevi" Bijeljina,
Republic of Srpska, Bosnia and Herzegovina

[2]University of East Sarajevo, Faculty of Medicine
Foča, Department of Primary Health Care and
Public Health,
Republic of Srpska, Bosnia and Herzegovina

[3]University of East Sarajevo, Faculty of Medicine
Foča, Department of Pharmacology,
Republic of Srpska, Bosnia and Herzegovina

[4]Public Health Institute of Republic of Srpska,
Bosnia and Herzegovina

[5]University of East Sarajevo, Faculty of Medicine
Foča, Department of Surgery,
Republic of Srpska, Bosnia and Herzegovina

[6]University hospital Foča,
Republic of Srpska, Bosnia and Herzegovina

[7]University of Belgrade, Faculty of Medicine,
Belgrade, Serbia

[8]University of East Sarajevo, Faculty of Medicine
Foča, Department of Preclinical Subjects
(Pathophysiology),
Republic of Srpska, Bosnia and Herzegovina

Corresponding author:
*Bojan Joksimović, MD, PhD
Studentska 5, 73300 Foča
bojannjoksimovic@gmail.com*

Summary

Introduction. Antibiotic resistance is a major threat to public health globally. The aim was to examine the impact of the COVID-19 pandemic on the distribution and antimicrobial resistance of pathogenic microorganisms isolated from samples obtained during standard hospital care in one hospital center.

Methods. Data were obtained retrospectively from a database of the hospital microbiology laboratory. Study sample consisted of 3012 samples tested before the pandemic in 2019 and 3130 samples from the pandemic period in 2021.

Results. There was no statistically significant difference in the occurrence of agents that were resistant to all antibiotics between the observed years, but there was the difference in the occurrence of those agents between departments, with the highest frequency in the intensive care unit and the COVID-19 department (p<0.001). Isolation of *Acinetobacter* bacteria increased 2.7 times, and *Clostridioides difficile* 6.4 times during 2021 compared to 2019. Statistically significant differences were registered in resistance to: imipenem, piperacillin-tazobactam, ceftayidime, cefepime, glycopeptides, aminoglycosides, levofloxacin and ciprofloxacin in 2021 compared to 2019.

Conclusion. Our results suggest possible influence of COVID-19 on antimicrobial resistance and input a need for a new larger study addressing this issue.

Keywords: antimicrobial resistance, COVID-19, multidrug-resistant bacteria

Introduction

Antibiotic resistance is a major threat to public health globally. Reports from the World Health Organization (WHO) indicate a high level of resistance (above 50%) in bacteria causing life-threatening blood infections, such as *Klebsiella pneumoniae* and *Acinetobacter* species, based on data reported by 87 countries in 2020. These infections require treatment with antibiotics of last resort, such as carbapenems. However, 8% of blood infections caused by *Klebsiella pneumoniae* have been reported to be resistant to carbapenems, which increases the risk of death. The same reports indicate that even common bacterial infections are becoming more resistant to treatments. Over 60% of *Neisseria gonorrhoeae* isolates showed resistance to one of the most commonly used oral antibacterial drugs, ciprofloxacin. Over 20% of *Escherichia coli* isolates were resistant to first-line drugs (ampicillin and co-trimoxazole) and to second-line therapy (fluoroquinolone) [1].

Antimicrobial resistance was related to nearly 5 million deaths in 2019. More than 2.8 million antimicrobial-resistant infections occur in the US each year, and more than 35,000 people die as a result of these infections. Adding *Clostridioides difficile*to, which is a bacterium that is typically not resistant but can cause fatal diarrhea and is related to antibiotic use, the number of all threats in the US exceeds 3 million infections and 48,000 deaths [2]. On average, 75% of COVID-19 cases worldwide were treated with antibiotics, while only 18% of patients who received antibiotics had secondary bacterial infections. On average, about half of the patients with COVID-19 who received antibiotics were neither severely nor critically ill [3]. A review of the literature by Segal et al indicated that several studies worldwide reported an unexpectedly high incidence of infections due to methicillin-resistant *Staphylococcus aureus*, carbapenem-resistant *Acinetobacter baumannii*, carbapenem-resistant *Enterobacteriacea*, and carbapenem-resistant *Klebsiella pneumoniae* and *Staphyloccocus aureus* among patients with COVID-19 in intensive care units [4]. At the University Hospital in Olomouc, a group of authors compared antibiotic resistance in intensive care patients in the pre-pandemic and pandemic periods. It was established that there was no change in the frequency of bacterial pathogens and their resistance to antibiotics during the COVID-19 pandemic. However, there was an increase or decrease in the percentage of some species and their resistance. The significant increase in resistance to piperacillin/tazobactam, cefotaxime, ciprofloxacin and gentamicin was found for *Serratia marcescens*. The reduction in the resistance of *Pseudomonas aeruginosa* and *Burkholderia cepacia* to meropenem was also determined [5].

The fear of secondary infections during the COVID-19 pandemic led to an escalation in the use of antibiotics worldwide. When it comes to the period of COVID-19, in the entire Republic of Srpska and in the Bijeljina region, the significant increase in the use of penicillin, macrolides and cephalosporins was registered compared to the period before pandemic [3]. It is not known if COVID-19 and increased antibiotic consumption in Republic of Srpska and Bijeljina Region had influence to antibiotic resistance.

The aim of this paper was to examine the differences in the distribution and antimicrobial resistance of pathogenic microorganisms isolated from samples obtained during standard hospital care of patients in the Public Health Institution "Sveti Vračevi" Hospital in Bijeljina, Republic of Srpska, Bosnia and Herzegovina (hereinafter Bijeljina Hospital) in 2019 (representing pre-pandemic period) and 2021 (representing pandemic period). The second aim was to consider possible influence of COVID-19 on observed differences.

Methods

Design and Data Collection

This retrospective study used data obtained from the database of the microbiology laboratory of Bijeljina Hospital. The study included 3012 samples, which were tested in the Microbiology Laboratory before the pandemic (from January 1 to December 31, 2019) and 3130 samples from the pandemic period (from January 1 to December 31, 2021). The study included patients with infection signs and symptoms as well as patients without apparent infection signs who underwent screening. Bijeljina Hospital provides secondary level health care for about 100,000 inhabitants. There are 294 beds, of which 284 are for acute treatment, and 10 intensive care beds. With the onset of the pandemic, 90 beds were made available in a special building of the hospital for the care of patients with COVID-19. The hospital annually has about 19,000 admissions, over 19,000 discharges and about 61,000 patient-days.

The hospital has a microbiology laboratory, which performs standard microbiological analyses, as well as PCR testing for SARS-CoV-2 and other pathogens.

Outcome Assessment

We compared the rate of positive isolates, the distribution of pathogenic microorganisms by type of sampled material and hospital departments where patients were treated, as well as rates of antimicrobial resistance for all isolated bacteria during 2019 and 2021.

Identification of isolated microorganisms

Identification of Enterobacteriaceae, Pseudomonas spp. and Acinetobacter spp. was based on the characteristics of growth on the substrates and the biochemical characteristics manifested in the biochemical series ("colorful series"). A classic biochemical sequence consisted of triple sugar, peptone water (indole test), Simmons citrate, mannitol agar, lactose, and urea.

Haemophilus influenzae was identified on the basis of growth on a selective medium for Haemophilus, requirement for V and X growth factor, phenomenon of satelliteism.

Gram-positive bacteria were identified based on the specificity of growth on the substrates and biochemical and antigenic characteristics: Staphylococcus spp. (coagulase test in a test tube), Streptococcus groups A, B, C, G (bacitracin test, detection of antigens by streptokit); Enterococcus spp. (esculin hydrolysis); Streptococcus pneumoniae (optochin test).

Identification was also performed using the Vitek2 system (bioMerieux) with the appropriate cards (GP ID, GN ID, ANC ID, NH ID,YST ID).

To identify the bacteria Serratia spp. we used the biochemical series and the Vitek2 system, cards for the identification of Gram-negative bacteria, GN ID.

The identification of the Burkholderia complex of bacteria was done in the Vitek2 system using cards for the identification of Gram-negative bacteria, GN ID.

Chromogenic agar CHROMID C.difficile, BioMerieux, was used for the detection and identification of Clostridioides difficile from stool samples, followed by a Gram preparation of grown colonies. Cultivation was done in an anaerobic environment, at the temperature of 35-37⁰C, for 48 hours. A rapid immunochromatographic test (Dialab, C. Difficile toxin A+B) was used to detect toxins A and B from stool.

Helicobacter pylori was detected from stool samples by a rapid immunochromatographic test (Artron HP antigen).

Detection and identification of the parasite Blastocystis hominis from stool samples was performed on the basis of morphological characteristics in a direct microscopic egsamation with physiological solution and Lugol.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the disk diffusion method according to EUCAST guidelines or by determining the MIC in the automated Vitek 2 system (bioMerieux) on cards intended for Gram-positive bacteria (AST P592, AST ST03) and Gram-negative bacteria (AST N222, AST N204, AST N 203). The inoculum was prepared according to the manufacturer's instructions for Vitek. Interpretation of MIC values and zones of inhibition obtained by the disc diffusion method were interpreted in accordance with the EUCAST guidelines:

S - Susceptible, standard dosing regimen: A microorganism is categorised as "Susceptible, standard dosing regimen", when there is a high likelihood of therapeutic success using a standard dosing regimen of the agent.

I - Susceptible, increased exposure: A microorganism is categorised as "Susceptible, Increased exposure*" when there is a high likelihood of therapeutic success because exposure to the agent is increased by adjusting the dosing regimen or by its concentration at the site of infection.

R - Resistant: A microorganism is categorised as "Resistant" when there is a high likelihood of therapeutic failure even when there is increased exposure.

Statistical Analysis

The methods of descriptive and analytical statistics were used for data description and analysis. Among the methods of descriptive statistics, measures of central tendency and measures of variability were used, namely: arithmetic mean with standard deviation. For the univariate analyses of possible differences in frequencies between groups nonparametric Chi-square test and Fisher test were used, and to determine the difference between groups in average values t test for independent samples was used. The usual value of $p < 0.05$ was taken as the level of statistical significance of differences. All statistical analyses were performed using IBM SPSS Statistics Software version 24.0 for Windows (IBM Corp., Armonk, NY, USA)

Results

The study included 6134 samples from hospitalized patients who were examined in the Microbiology Laboratory of Bijeljina Hospital, of which 3011 samples (48.2 samples per 1000 patient days) in 2019, and 3123 samples (47.0 samples per 1000 patient days) in 2021. In 2019, the samples came from female patients statistically significantly more often, while the distribution by sex in 2021 was approximately the same. The average age of the patients was 51.92 years and the patients in 2021 were statistically significantly older than those in 2019 (43.62 years) ($p<0.001$). The largest number of samples were sent to the laboratory from the department of internal medicine, then from the department for the care of patients with COVID-19 and from the pediatric department, while the smallest number of samples were sent from the ophthalmology department ($p<0.001$). There was no significant difference in the number of culture positive specimens before and during COVID-19 (40.4% vs. 40.1%). The distribution of different types of culture positive specimens is shown in table 1 and table 2.

Table 2 shows the descriptive analyses of patients with culture positive specimens. Culture positive specimens in 2021 were dominantly from males (48.1%) when compared to 2019 (40.8%) ($p<0.001$). Also, patients with culture positive specimens were significantly older in 2021 (54.98±26.68 years) when compared to the patients in 2019 (43.16±28.21 years) (Table 2).

Table 1. Descriptive analyses of patients

Variables	2019 (n=3011) n (%)	2021 (n=3123) n (%)	p
Sex			
Male	1299 (43.1)	174 (50.3)	<0.001*
Female	1713 (56.9)	1556 (49.7)	
Age, years	43.62±28.51	51.92±30.02	<0.001**
Departments			
Infectology	15 (0.5)	5 (0.2)	
Internal medicine	890 (29.6)	1003 (32.1)	
Surgery	455 (15.1)	311 (10.0)	
Pulmology	164 (5.4)	96 (3.1)	
Pediatrics	725 (24.1)	498 (15.9)	
Intensive care unit	156 (5.2)	30 (1.0)	<0.001*
Gynecology	446 (14.8)	290 (9.3)	
Neurology	93 (3.1)	61 (2.0)	
Ophtalmology	4 (0.1)	8 (0.3)	
ORL	62 (2.1)	54 (1.7)	
COVID-19	0 (0.0)	768 (24.5)	
Culture positivity			
Positive	1217 (40.4)	1255 (40.1)	0.066*
Negative	1795 (59.6)	1876 (59.9)	
Type of culture positive specimen			
Urine	525 (43.1)	629 (50.1)	
Blood	50 (40.1)	91 (7.3)	
Smear of wound	119 (9.8)	58 (4.6)	
Smear of nose/throat	57 (4.7)	13 (1.0)	
Drain content	20 (1.7)	14 (1.1)	
Cervical smear	144 (11.8)	55 (4.4)	
Stool	94 (7.7)	239 (19.0)	<0.001*
Sputum	64 (5.3)	21 (1.7)	
Vaginal smear	8 (0.7)	5 (0.4)	
Eye smear	18 (1.5)	14 (1.1)	
Tracheal aspirate	34 (2.8)	31 (2.5)	
Bronchial aspirate	13 (1.1)	18 (1.4)	
Smear***	66 (5.4)	57 (4.5)	
Central venous catheter	5 (0.4)	6 (0.5)	

ORL - Otorhinolaringology; *Chi-square test; **Wilcoxon Rank Sum Test; p - statistical significance; ***screening smear in patients who have been transferred from another health institution

Table 2. Descriptive analyses of patients with culture positive specimens

Variables	2019 (n=1217) n (%)	2021 (n=1255) n (%)	p
Sex			
Male	496 (40.8)	604 (48.1)	<0.001*
Female	721 (59.2)	651 (51.9)	
Age, years	43.16±28.21	54.98±26.68	<0.001**

*Chi-square test; **Wilcoxon Rank Sum Test; p - statistical significance

The most frequently isolated infectious agent was *Escherichia coli*, both in 2019 and in 2021, accounting for 13.2% of the total number of isolates. This number was followed by *Enterococcus* species (9.5%), *Candida* species (8.6%), *Klebsiella/Enterobacter* (7.8%) and CoNS (7.8%). During 2021, there was the increase in the frequency of a larger number of isolates, of which the largest increase was recorded in *Clostridioides difficile*. This bacterium was

isolated more frequently in 2021 compared to 2019. The ratios of the numbers and percentages of samples with *Cl. difficile* in 2021 compared to those in 2019 were 6.6 and 6.2, respectively. *Acinetobacter baumannii* isolates were 2.7 times more frequent in 2021 compared to 2019. The percentage of this bacterium among all isolates was 2.6 times higher than in 2021. Both *Klebsiella/Enterobacter* and *Enterococcus* were isolated 1.2 times more frequently in 2021 than in 2019 (Table 3).

Table 3. The frequency of positive cultures of infectious agents in the analyzed samples

Microorganisms	2019 (n=1219) n (%)	2021 (n=1258) n (%)	Total (n=2477) n (%)	p	OR	95% CI Lower	95% CI Upper
Staphylococcus aureus	63 (5.2)	39 (3.1)	102 (4.1)	0.012**	1.694	1.132	2.533
Klebsiella/Enterobacter	88 (7.2)	106 (8.4)	194 (7.8)	0.308**	0.859	0.644	0.154
Enterococcus spp	105 (8.6)	131 (10.4)	236 (9.5)	0.163**	0.827	0.637	1.075
Pseudomonas aeruginosa	64 (5.3)	35 (2.8)	99 (4.0)	0.002*	1.920	1.268	2.908
E. Coli	175 (14.4)	153 (12.2)	328 (13.2)	0.112*	1.201	0.961	1.500
CoNS	118 (9.7)	76 (6.0)	194 (7.8)	0.001*	1.639	1.223	2.196
Candida spp.	98 (8.0)	116 (9.2)	214 (8.6)	0.335*	0.874	0.665	1.149
Proteus mirabilis	51 (4.2)	48 (3.8)	99 (4.0)	0.618**	1.106	0.744	1.646
Haemophilus influenzae	3 (0.2)	2 (0.2)	5 (0.2)	0.682**	1.560	0.260	9.341
Streptococcus spp.	45 (3.7)	15 (1.2)	60 (2.4)	<0.001**	3.151	1.753	5.664
Acinetobacter baumannii	19 (1.6)	51 (4.1)	70 (2.8)	<0.001**	0.383	0.226	0.651
Streptococcus pyogenes	10 (0.8)	2 (0.2)	12 (0.5)	0.020**	5.212	1.141	23.805
Citrobacter spp.	4 (0.3)	2 (0.2)	6 (0.2)	0.444**	2.080	0.381	11.367
Streptococcus pneumonia	5 (0.4)	4 (0.3)	9 (0.4)	0.749**	1.300	0.349	4.845
Campilobacter spp.	7 (0.6)	8 (0.6)	15 (0.6)	1.000**	0.909	0.329	2.511
Salmonella enteritidis	3 (0.2)	12 (1.0)	15 (0.6)	0.035**	0.259	0.073	0.919
Corynebacterium spp.	0 (0.0)	2 (0.2)	2 (0.1)	0.500**	1.963	1.915	2.011
Morganella Morganii	3 (0.2)	2 (0.2)	5 (0.2)	0.682**	1.560	0.260	9.341
Blastocystis hominis	2 (0.2)	2 (0.2)	4 (0.2)	1.000**	1.040	0.146	7.384
Serratia spp.	6 (0.5)	1 (0.1)	7 (0.3)	0.065**	6.248	0.752	51.924
Clostridioides difficile	21 (1.7)	134 (10.6)	155 (6.3)	<0.001**	0.157	0.099	0.249
Multidrug-resistant bacteria (MRSA, VRE, CARBA, ESBL)	17 (1.4)	22 (1.7)	39 (1.6)	0.524**	0.802	0.425	1.514
Burkholderia	0 (0.0)	1 (0.1)	1 (0.0)	1.000**	1.962	1.915	2.011
Helicobacter pylori	0 (0.0)	1 (0.1)	1 (0.1)	1.000**	1.962	1.915	2.011
More isolates	312 (25.6)	293 (23.3)	605 (24.4)	0.261*	1.109	0.929	1.322

MRSA - Methicillin-resistant *Staphylococcusaureus,* VRE - vancomycin-resistant *Enterococcus* spp., ESBL - extended-spectrum beta-lactamase producers, CARBA - carbapenemase-resistant Enterobacteriaceae; *Chi-square test test; **Fisher test p - statistical significance; OR - adjusted odds ratio; CI - confidence interval

The analysis of the frequency of pan-resistant bacteria resistant to all tested antibiotics indicated that there was no statistically significant difference in their frequency comparing 2019 and 2021 (p=0.567). It could be seen that their frequency was even higher in 2019 (2.4%) compared to 2021 (1.9%) (Table 4).

Table 4. Frequency of pan-resistant isolates in the overall structure of isolates

Pan-Resistant	2019 n (%)	2021 n (%)	Total n (%)	p	OR	95% CI Lower	95% CI Upper
No	647 (97.6)	605 (98.1)	1252 (97.8)	0.567*			
Yes	16 (2.4)	12 (1.9)	28 (2.2)		1.852	1.813	2.269

*Chi-square test test; p - statistical significance; OR - adjusted odds ratio; CI - confidence interval

The analysis of the frequency of pan-resistant isolates by departments of Bijeljina Hospital during 2021 indicated the highest frequency in the department for the care of patients with COVID-19, and pan-resistant isolates were also registered in the departments of pediatrics and internal medicine. Differences in the frequency of pan-resistant isolates between departments of Bijeljina Hospital were statistically significant (p<0.001) (Table 5).

Table 5. Frequency of pan-resistant isolates during 2021 by departments

| Departments | 2021 n (%) | | Total n (%) | p | OR | 95% CI | |
	No (n=605)	Yes (n=12)				Lower	Upper
Infectology	2 (0.3)	0 (0.0)	2 (0.3)				
Internal medicine	203 (32.9)	3 (1.5)	206 (33.4)				
Surgery	68 (11.0)	0 (0.0)	68 (11.0)				
Pulmology	22 (3.6)	0 (0.0)	22 (3.6)				
Pediatrics	86 (13.9)	3 (0.5)	89 (14.4)				
Intensive care unit	7 (1.1)	0 (0.0)	7 (1.1)	<0.001*	1.652	1.083	2.658
Gynecology	37 (6.0)	0 (0.0)	37 (6)				
Neurology	14 (2.3)	0 (0.0)	14 (2.3)				
Ophtalmology	0 (0.0)	0 (0.0)	0 (0.0)				
ORL	3 (0.5)	0 (0.0)	3 (0.5)				
COVID-19	163 (26.4)	6 (3.6)	169 (27.4)				

ORL - Otorhinolaringology; *Chi-square test; p - statistical significance; OR - adjusted odds ratio; CI - confidence interval

In addition to the fact that the increased frequency of *Acinetobacter baumannii* isolates was registered during 2021 compared to 2019, the increase in the resistance of this bacterium to carbapenems was also registered. The high statistically significant difference was registered in the rate of resistance of *Acinetobacter baumannii* to imipenem and meropenem in 2021 compared to 2019 (p=<0.001), which is shown in table 6.

Table 6. Resistance of *Acinetobacter baumannii* bacteria to carbapenems

| Carbapenems | 2019 n of resistant/n of isolated bacteria, (%) | 2021 n of resistant/n of isolated bacteria, (%) | Total n of resistant/n of isolated bacteria, (%) | p | OR | 95% CI | |
						Lower	Upper
Imipenem	11/19 (57.9)	40/51 (78.4)	51/70 (72.9)	<0.001*	2.972	1.743	4.831
Meropenem	13/19 (68.4)	41/51 (80.4)	54/70 (77.1)	<0.001*	2.439	1.659	3.793

*Chi-square test; p - statistical significance; OR - adjusted odds ratio; CI - confidence interval

The resistance of *Pseudomonas aeruginosa* to carbapenems (meropenem and imipenem) decreased in 2021. The difference in resistance to imipenem was not statistically significant (p=0.090), while the difference in resistance to meropenem was significant (p=0.030) (Table 7).

Table 7. Resistance of *Pseudomonas aeruginosa* to carbapenems

Carbapenems	2019 n of resistant/n of isolated bacteria, (%) n (%)	2021 n of resistant/n of isolated bacteria, (%)	Total n of resistant/n of isolated bacteria, (%)	p	OR	95% CI	
						Lower	Upper
Imipenem	9/64 (14.0%)	3/35 (8.6%)	12/99 (12.1%)	0.090*	2.080	1.092	2.758
Meropenem	12/64 (18.8%)	5/35 (14.3)	17/99 (17.2%)	0.030*	0.735	0.361	1.892

*Fisher test; p - statistical significance; OR - adjusted odds ratio; CI - confidence interval

The rate of *Klebsiella*/*Enterobacter* resistance increased in 2021 compared to 2019 for imipenem, meropenem and aikacin but this difference was not statistically significant. The levofloxacin resistance rate decreased in 2021 compared to 2019, but this difference was not statistically significant (Table 8).

Table 8. Resistance of *Klebsiella*/*Enterobacter* bacteria to carbapenems, aminoglycosides and quinolones

Carbapenems, aminoglycosides and quinolones	2019 n of resistant/n of isolated bacteria, (%) n (%)	2021 n of resistant/n of isolated bacteria, (%) n (%)	Total n of resistant/n of isolated bacteria, (%) n (%)	p	OR	95% CI	
						Lower	Upper
Imipenem	32/88 (36.4%)	56/106 (52.8%)	88/194 (45.4)	0.072*	1.102	0.581	2.283
Meropenem	39/88 (44.3%)	54/106 (50.9)	93/194 (47.9)	0.208*	1.370	0.788	1.953
Amikacin	62/88 (70.5%)	81/106 (76.4%)	143/194 (73.7)	0.470*	0.936	0.412	1.994
Levofloxacin	79/88 (89.8%)	92/106 (86.8%)	171/194 (88.1)	0.536*	0.760	0.337	2.103

*Fisher test; p - statistical significance; OR - adjusted odds ratio; CI - confidence interval

The analysis of the resistance of all infectious agents to antibiotics by species and groups indicates that differences were registered during 2021 compared to 2019.

The frequency of resistance of infectious agents to piperacillin/tazobactam decreased during 2021 compared to 2019 (p<0.001). Differences were also registered in relation to third generation cephalosporins. Resistance to ceftazidime decreased in 2021 compared to 2019 (p<0.001), while resistance to other third-generation cephalosporins remained at the same level. The resistance of pathogens to fourth-generation cephalosporins (cefepime) decreased during 2021 compared to 2019 (p=0.021). The increase in resistance to carbapenems (Imipenem) was registered in 2021, which was statistically significant (p<0.001). There was also the significant increase in the resistance of infectious agents to glycopeptide antibiotics (vancomycin) during 2021 (p=0.024). The frequency of resistance to aminoglycosides (gentamicin and amikacin) was increased (p<0.001). When it comes to quinolone antibiotics, resistance increased regarding levofloxacin and ciprofloxacin (p<0.001). The resistance of infectious agents to antibiotics by group and species is shown in Table 9.

Table 9. Resistance of infectious agents to antibiotics by species and groups in 2019 and 2021

Antibiotics	2019 n (%)	2021 n (%)	Total n of analyzed/n of resistant (%)	p	OR	95% CI	
						Lower	Upper
Piperacillin tazobactame	52 (38.8)	32 (35.6)	224/84 (37.5)	<0.001*	1.910	0.783	2.835
Cephalosporins of III generation							
Ceftriaxone	164 (58.8)	125 (59.0)	491/289 (58.9)	0.067*	1.327	1.002	4.286
Cefotaxime	31 (88.6)	42 (89.4)	82/73 (89.0)	0.162*	1.338	0.255	2.348
Ceftazidime	64 (72.7)	50 (60.2)	171/114 (66.7)	<0.001*	4.375	1.131	14.297
Cephalosporins of IV generation							
Cefepime	23 (85.2)	11 (68.8)	43/34 (79.1)	0.021*	0.812	0.287	1.481
Carbapenems							
Imipenem	36 (21.7)	57 (39.6)	310/93 (30.0)	<0.001*	4.385	1.492	8.289
Meropenem	46 (30.1)	58(43.0)	288/104 (36.1)	0.065*	2.157	1.204	3.208
Glycopeptides							
Vancomycine	3 (7.7)	12 (26.7)	84/15 (17.9)	0.024*	3.021	1.579	5.997
Teicoplanine	1 (100.0)	0 (0.0)	5/1 (20.0)	0.025**	2.672	0.985	6.239
Amynoglycosides							
Gentamycine	221 (42.9)	272 (58.4)	981/493 (50.3)	<0.001*	3.558	1.496	4.991
Amicacin	81 (28.0)	100 (44.1)	516/181 (35.1)	<0.001*	2.017	0.965	5.208
Quinolones							
Levofloxacin	99 (61.9)	148 (75.9)	355/247 (69.6)	<0.001*	3.009	1.887	6.308
Ciprofloxacin	155 (47.1)	186 (59.8)	640/341 (53.3)	<0.001*	2.887	1.054	3.783
Norfloxacin	158 (57.5)	176 (62.9)	555/334 (60.2)	0.369*	2.100	1.285	4.209
Moxifloxacin	11 (39.3)	9 (56.3)	44/20 (45.5)	0.179*	1.023	0.558	2.852

* Chi-square test; **Fisher test p – statistical significance; OR – adjusted odds ratio; CI – confidence interval.

Discussion

The COVID-19 pandemic had the significant impact on the distribution and antimicrobial resistance of pathogenic microorganisms isolated from samples obtained during standard hospital care of patients in the Public Health Institution "Sveti Vračevi" Hospital in Bijeljina, Republic of Srpska, Bosnia and Herzegovina, with the increase in isolates and resistance of multiresistant bacterium *Acinetobacter baumannii*. The number of patients treated in the hospital was higher in 2021 than in 2019, which was a consequence of the COVID-19 pandemic. The distribution of patients by departments during these two years differed, mostly due to the large number of patients who were hospitalized in the COVID department during 2021. The distribution of patients by sex indicated the statistically significantly higher number of women who were treated in the hospital before the pandemic, while during the pandemic the distribution by sex did not differ significantly. Sex differences probably arose due to the fact that women reported their health problems more often than men, not only in BiH but in the whole Europe[6]. According to official data, from the beginning of the pandemic till today, infection with the SARS-CoV-2 virus has been confirmed in 121,009 people (26.49% of all tested), of which 58,905 were males and 62,104 were females. This difference in favor of women is probably a consequence of women's greater health responsibility and a higher probability of undergoing testing [7].

The number of isolates for microbiological analysis in relation to the number of patient-days did not change during the pandemic period compared to the period before. The number of isolates from patients with infection signs and symptoms as well as from

screened patients without apparent infections signs were at the same level during both observed periods. Sampling remained at a satisfactory level, i.e. almost five times more frequent than in the Doboj hospital within the same country [8].

The most frequently isolated pathogen during both observed years was *Escherichia coli*, and its frequency did not change significantly. However, the increase in the frequency of isolation of *Acinetobacter*, one of the multiresistant hospital strains, was registered. Also, the increases of isolation of Streptococcus spp. and Streptococcus pyogenes were observed. It should be further considered with the maximal care because it is suggested that Streptococcus spp. are common cause of respiratory infections [4].

Also, in 2021, there was the increase in the number of clostridial infections that globally represent the most common hospital infections. Clostridial infections (CDI) have become a worrisome health problem in recent decades. Diarrhea during COVID-19 occurred in some patients as a part of the clinical presentation of the disease. However, the overuse of broad-spectrum antibiotics, observed during COVID-19, may favor the occurrence of diarrhea of other etiologies such as CDI [9-11]. Many studies have shown the significant increase in the incidence of clostridial infections during the COVID-19 pandemic compared to the pre-pandemic period, from 2.6% to 10.9% [12]. Very similar results were found in Bijeljina hospital, where the frequency of clostridial infections increased from 1.7% to 10.6%. This increase can be explained by repeated and prolonged hospitalizations of patients posing the significant risk factor for clostridial infections, as well as increased use of antibiotics [12, 13]. Lewandowski at al [12] showed that the risk of acquiring CDI increased by 3% with each day of hospitalization. It is also assumed that the large outpatient consumption of antibiotics in the Bijeljina region certainly contributed to the increase in the number

of clostridial infections [13]. Glucocorticoid therapy is also recognized as the significant risk factor for the occurrence of CDI in the population of patients with COVID-19 [14]. In the recommendations for the treatment of patients with COVID-19, corticosteroids were among the drugs of choice, which, together with the lack of specific antiviral drugs, led to their widespread use and could have contributed to the increase in the frequency of CDI.

Frequancies of isolation of Staphylococcs aureus, CoNS and Pseudomonas aeruginosa declined in 2021 compared to 2019. This result is in accordance with the finding of the Hirabayashi et al [15] who analyzed results from more than 1300 hospitals in 2019 and 2020.

The major health problems today are infections caused by *Acinetobacter baumannii* due to its high rate of resistance to antibiotics. *Acinetobacter baumannii* is a Gram-negative aerobic coccobacillus. It belongs to the "ESCAPE" group of pathogens with multiple resistance and virulence. The high mortality rate caused by this bacterium due to its resistance to the large number of antibiotics represents a serious problem, and a challenge for doctors and health systems. Increasing prevalence of *Acinetobacter baumannii* resistance to carbapenems is caused by genetic elements such as transposons, plasmids or integrons and cassettes [4]. In Bijeljina hospital, especially in the COVID department, the statistically significant increase in the isolation of *Acinetobacter* was registered. The fact that the rate of *Acinetobacter* resistance to carbapenems increased in 2021 compared to the period before the pandemic is an additional concern. This trend has been confirmed in other hospitals in the world and in Bosnia and Herzegovina [4, 8]. In the same source, it is stated that 80.5% of non-fermentative Gram-negative bacilli were resistant to carbapenems. If we take into account that this study was conducted more than five years ago, it can be concluded that the resistance of *Acinetobacter baumannii* bacteria to imipenem of 78.4% and meropenem

of 80.4% is more favorable than in neighboring Serbia, but it is also high in our country.

AMR was very high for most pathogens - 80.5% of non-fermenting Gram-negative bacilli were resistant to carbapenems.

Pseudomonas aeruginosa resistance to carbapenems decreased during the pandemic period. We hypothesize that improved hygiene measures, including increased use of personal protective equipment, have led to reduced contact transmission and nosocomial spread of *Pseudomonas aeruginosa* bacteremia. Data related to the impact of the COVID-19 pandemic on the incidence of bacteremias caused by *Pseudomonas aeruginosa* are contradictory, since there are studies that show the increase, but also those in which the rate of these incidences decreased or remained at the same level as before the pandemic [16].

When it comes to other multiresistant strains, there was no significant change in the rates of isolation and resistance to antibiotics. This is partially consistent with the results of Segala et al [4], who, by reviewing the literature, showed that global resistance to MRSA, CARBA decreased or remained unchanged during the COVID-19 pandemic compared to earlier period, while the increase in resistance to VRE was registered in Germany, Italy and Spain.

In order to mitigate the potential long-term impact of COVID-19 on AMR, it is necessary to integrate antimicrobial management activities in the coming period. One of the reasons for increased antibiotic prescribing must have been limited knowledge of the clinical manifestations of COVID-19. The observed rates of bacterial superinfection in patients with COVID-19 as well as their causal pathogens were different compared to other respiratory illnesses such as influenza.

The conducted study is descriptive with a retrospective design. In addition, the collected data were not obtained from the epidemiological surveillance of hospital infections, but from the protocol of the Microbiology Laboratory. Therefore, it is not known for each infection whether it is the hospital infection, or whether the patient with the infection came from the community. The aforementioned study design did not allow us to examine the risk factors leading to antibiotic resistance. Despite the large number of samples, the number of isolates was small, which could affect the relevance of the conclusions.

Conclusions

The SARS-COVID-19 pandemic led to a massive disruption of the health system at all levels. In conditions of acute and intensive care, the inappropriate use of antimicrobial drugs and interruption of infection control measures can lead to the selection and spread of antibiotic-resistant pathogens. Beside differences in frequencies of isolation of several pathogens, statistically significant differences in their resistance to: imipenem, piperacillin-tazobactam, ceftazidime, cefepime, glycopeptides, aminoglycosides, levofloxacin and ciprofloxacin in 2021 compared to 2019 were registered. Our results suggest to possible influence of COVID-19 to antimicrobial resistance. Due to the increase in the consumption of antibiotics during the pandemic, there was the increase in the isolation of *Clostridioides difficile*. Due to all of the above, it is necessary to conduct prospective studies dealing with the issue of antibiotic resistance, with a special emphasis on COVID-19 which can be a new trigger of AMR. Antimicrobial resistance prevention strategies should also be developed for future pandemics caused by similar viruses. Antimicrobial resistance will continue to represent the significant threat to the health system, especially in the near future, so it is necessary to develop protocols for the management of antimicrobial drugs in pandemic conditions.

References:

1. World Health Organization. Report signals increased resistance to antibiotic in bacterial infections in humans and need for better data 2022 [cited 2023 24.03]. Available from: https://www.who.int/news/item/09-12-2022-report-signals-increasing-resistance-to-antibiotics-in-bacterial-infections-in-humans-and-need-for-better-data

2. Center for Disease Control and Prevention: National Infection & Death Estimates for Antimicrobial Resistance. 2021 [cited 2023 24.03]. Available from: https://www.cdc.gov/drugresistance/national-estimates.html

3. Chedid M, Waked R, Haddad E, Chetata N, Saliba G, Choucair J. Antibiotics in treatment of COVID-19 complications: a review of frequency, indications, and efficacy. J Infect Public Health 2021;14(5):570–6.

4. Segala FV, Bavaro DF, Di Gennaro F, Salvati F, Marotta C, Saracino A, et al. Impact of SARS-CoV-2 Epidemic on Antimicrobial Resistance: A Literature Review. Viruses 2021;13(11)2110.

5. Fišerová K, Doubravská L, Htoutou Sedláková M, Kolář M. [Impact of the COVID-19 pandemic on antimicrobial resistance]. Klin Mikrobiol Infekc Lek 2022;28(2):36–41.

6. Oksuzyan A, Gumà, J, Doblhammer G. A demographic perspective on gender, family and health in Europe. In: Doblhammer G, Gumà J, editors. Sex differences in health and survival. Cham, Switzerland: Springer Nature; 2018. p. 65–100.

7. Srpska IfPHotRo. COVID-19: Epidemiological situation in the Republic of Srpska, March 29, 2023 at 9 a.m. 2023. Available from: https://www.phi.rs.ba/index.php?view=clanak&id=2834&lang=SR-CIR

8. Sokolović D, Drakul D, Vujić-Aleksić V, Joksimović B, Marić S, Nežić L. Antibiotic consumption and antimicrobial resistance in the SARS-CoV-2 pandemic: A single-center experience. Front Pharmacol 2023;14:1067973.

9. Lakkasani S, Chan KH, Shaaban HS. Clostridioides difficile in COVID-19 Patients, Detroit, Michigan, USA, March-April 2020. Emerg Infect Dis 2020;26(9):2299–300.

10. Han C, Duan C, Zhang S, Spiegel B, Shi H, Wang W, et al. Digestive Symptoms in COVID-19 Patients With Mild Disease Severity: Clinical Presentation, Stool Viral RNA Testing, and Outcomes. Am J Gastroenterol 2020;115(6):916–23.

11. Guh AY, Mu Y, Winston LG, Johnston H, Olson D, Farley MM, et al. Trends in U.S. Burden of Clostridioides difficile Infection and Outcomes. N Engl J Med 2020;382(14):1320–30.

12. Lewandowski K, Rosołowski M, Kaniewska M, Kucha P, Meler A, Wierzba W, et al. Clostridioides difficile infection in coronavirus disease 2019 (COVID-19): an underestimated problem? Pol Arch Intern Med 2021;131(2):121–7.

13. Sokolović D, Drakul D, Joksimović B, Lalović N, Avram N, Milić M, et al. Consumption of Antibiotics in Primary Care Setting before and during COVID-19 Pandemic in Republic of Srpska, Bosnia and Herzegovina. Antibiotics (Basel) 2022;11(10):1319.

14. Granata G, Petrosillo N, Al Moghazi S, Caraffa E, Puro V, Tillotson G, et al. The burden of Clostridioides difficile infection in COVID-19 patients: A systematic review and meta-analysis. Anaerobe 2022;74:102484.

15. Hirabayashi A, Kajihara T, Yahara K, Shibayama K, Sugai M. Impact of the COVID-19 pandemic on the surveillance of antimicrobial resistance. J Hosp Infect 2021;117:147–56.

16. Ng QX, Ong NY, Lee DYX, Yau CE, Lim YL, Kwa ALH, et al. Trends in Pseudomonas aeruginosa (P. aeruginosa) Bacteremia during the COVID-19 Pandemic: A Systematic Review. Antibiotics (Basel) 2023;12(2):409.

Uticaj pandemije COVID-19 na antimikrobnu rezistenciju na nivou opšte bolnice u Republici Srpskoj, Bosna i Hercegovina

Nasta Manojlović[1], Biljana Mijović[1,2,6], Zlatko Maksimović[1], Dragana Sokolović[3], Sanda Lazić[1], Ljubica Bojanić[4], Dragana Drakul[3], Nenad Lalović[5,6] Marija Lukić[7], Bojan Joksimović[8]

[1]JZU Bolnica "Sveti vračevi" Bijeljina, Republika Srpska, Bosna i Hercegovina

[2]Univerzitet u Istočnom Sarajevu, Medicinski fakultet Foča, Katedra za primarnu zdravstvenu zaštitu i javno zdravlje, Republika Srpska, Bosna i Hercegovina

[3]Univerzitet u Istočnom Sarajevu, Medicinski fakultet Foča, Katedra za farmakologiju, Republika Srpska, Bosna i Hercegovina

[4]Institut za javno zdravstvo Republike Srpske, Republika Srpska, Bosna i Hercegovina

[5]Univerzitet u Istočnom Sarajevu, Medicinski fakultet Foča, Katedra za hirurgiju, Republika Srpska, Bosna i Hercegovina

[6]Univerzitetska bolnica Foča, Republika Srpska, Bosna i Hercegovina

[7]Univerzitet u Beogradu, Medicinski fakultet, Beograd, Srbija

[8]Univerzitet u Istočnom Sarajevu, Medicinski fakultet Foča, Katedra za pretkliničke predmete (patofiziologija), Republika Srpska, Bosna i Hercegovina

Uvod. Antimikrobna rezistencija predstavlja globalno veliku prijetnju za javno zdravlje. Cilj studije bio je ispitati uticaj pandemije COVID-19 na distribuciju i antimikrobnu rezistenciju patogenih mikroorganizama izolovanih iz uzoraka dobijenih tokom standardne bolničke njege u jednom bolničkom centru.

Metode. Podaci su dobijeni retrospektivno iz baze podataka laboratorije za mikrobiologiju bolnice. Uzorak studije je obuhvatao 3.012 uzoraka testiranih prije pandemije 2019. godine i 3.130 uzoraka iz perioda pandemije, 2021. godine.

Rezultati. Nije zabilježena statistički značajna razlika u pojavi agenasa rezistentnih na sve antibiotike između posmatranih godina, ali je postojala razlika u pojavi ovih agenasa između odjeljenja, sa najvećom učestalošću u jedinici intenzivne njege i COVID-19 odjeljenju ($p < 0,001$). Izolacija bakterija *Acinetobacter* povećana je 2,7 puta, a *Clostridioides difficile* 6,4 puta tokom 2021. u poređenju sa 2019. godinom. Statistički značajne razlike su zabilježene u rezistenciji na: imipenem, piperacilin-tazobaktam, ceftazidim, cefepim, glikopeptide, aminoglikozide, levofloksacin i ciprofloksacin u 2021. u poređenju sa 2019. godinom.

Zaključak. Naši rezultati ukazuju na mogući uticaj COVID-19 na antimikrobnu rezistenciju i naglašavaju potrebu za novom, većom studijom koja će se baviti ovim pitanjem.

Ključne riječi: antimikrobna rezistencija, COVID-19, multirezistentne bakterije