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Original Scientific Paper

COMPARISON OF TRACHEAL ASPIRATES IN THE PERIOD BEFORE AND AFTER THE START OF THE COVID-19 PANDEMIC IN THE INTENSIVE CARE UNIT IN A TERTIARY HOSPITAL

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SUMMARY – Changes in working methods and diagnostics using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) diagnostics that occurred after the start of the COVID-19 pandemic could show differences in the prevalence of positive microbiological samples. In a retrospective study, a total of 442 tracheal aspirates in the pre-pandemic period (Period A, 2018, 198 patients, age median 69 (57-78)) and 277 samples in the pandemic period (Period B, 2021, 147 patients, age 68 (56-77) (p=0.585) obtained after the start of the pandemic were analyzed. A total of 176 patients had at least 1 positive result. In Period A, there were 245 (55%) and in Period B 186 (68%) sterile samples (p=0.001). The most frequently isolated pathogens were *Acinetobacter baumannii* in 86 patients from Period A and 32 patients from Period B, i.e., 43% *vs*. 21.7% of all positive isolates (p=0.247), followed by *Pseudomonas aeruginosa* in 29 patients in Period A (14.6%) *vs*. 7 (3%) (p=0.112) in Period B. A statistically significant increase was observed in the incidence of *Enterobacterales* (16.6% *vs*. 32.6%, p=0.002), especially *Klebsiellae* spp. Although overall mortality decreased in Period B, changes in the working methods and diagnostics did not result in changes in the mortality of patients whose tracheal aspirates were sampled.

Key words: *Spectrometry, mass; Matrix-assisted laser desorption-ionization; Pneumonia; Intensive care units; Microbiology*

Introduction

Treatment and care of critically ill or polytraumatized patients takes place in the most sophisticated units in hospitals, intensive care units (ICU). Medical care for seriously ill patients consists of creating treatment strategies, creating and applying treatment protocols, monitoring treatment effectiveness, and conducting scientific research, with special equipment and particularly trained staff[1]. Mechanical ventilation is one of the indications for admitting the patient to the ICU. The standard criteria for mechanical ventilation are acute respiratory insufficiency, threatening respiratory insufficiency, hypoxemic respiratory insufficiency with increased work of breathing, cardiopulmonary resuscitation, postoperative period, sepsis, increase in intracranial pressure, Glasgow Coma Score (GCS)

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≤8, and severe burns of the face and neck with possible swelling of the airway[2]. When using mechanical ventilation, the airway is usually secured with an orotracheal tube, less often with a tracheal cannula. However, infection and sepsis significantly correlate with death in many ICUs, and ventilator-associated pneumonia plays a huge role in predicting outcome since even 10%-20% of patients who are mechanically ventilated for more than 48 hours develop pneumonia[3]. Microbiological analysis of tracheal aspirates is used to guide treatment of pneumonia to adapt antibiotic therapy to a specific pathogen. Direct microbiological sampling of tracheal aspirates, either by aspiration or by bronchoscopy, is recommended before the introduction of initial antibiotic therapy[3]. The technology that has been used for the last ten years in the world is matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), which is useful not only in the identification of pathogens but also in the optimization of antibiotic and antifungal therapy[4].

In 2020, a disease caused by the new coronavirus appeared all over the world and thus greatly changed both everyday life and the entire healthcare system. The COVID-19 pandemic has changed aspects of treatment in ICUs by improving awareness of the spread of infection and the importance of protective equipment. The use of surgical masks and disposable gloves in combination with good hand hygiene in ICUs even with managing COVID-19 negative patients could have positive impact on preventing the spread of intrahospital infections, as shown in the study from Kuwaiti teaching hospital, where they showed that good hand hygiene had an impact on cross contamination in the ICU[5].

With the onset of the COVID-19 pandemic, numerous institutions have redirected staff from ICUs to COVID-ICUs. Due to the needs of this organization, a large number of new staff were involved in the work of ICUs, and some of previous staff members moved to other wards due to burnout. This staff turnover could also result in suboptimal patient care and more infections.

It is likely that after the start of the COVID-19 pandemic, patients have been admitted to the hospital with more advanced diseases. Underlying reasons may be that the flow of patients through the health system is slower due to the repeated testing, common delays due to isolations, and due to changes in the healthcare organization. In surgical ICU patients, this could result in a higher number of pneumonias caused by resistant pathogens, increased mortality, and prolonged mechanical ventilation time.

The aim of this study was to compare comorbidity, laboratory indicators, microbiological indicators and outcomes of patients treated in the ICU of the tertiary Osijek University Hospital in the period before the start of the COVID-19 pandemic and in the period after the start of the pandemic.

Material and Methods

In this study, two groups of patients who had tracheal aspirate samples obtained for microbiological analysis in 2018 (Period A) and 2021 (Period B) during ICU treatment were included. All data on microbiological samples were collected from the Department of Intensive Care Medicine database, patient medical charts, and hospital database. The number of polymorphonuclear neutrophils (PMN) in the samples was shown in ranks as described by the microbiology laboratory. Number 1 is displayed when no PMN was found; 2 denotes <5; 3 denotes 5-10; 4 denotes <10; 5 denotes 10-15; 6 denotes 10-25; 7 denotes 15-20; 8 denotes 20-25; and 9 denotes >25 PMNs in the field. The number of bacteria was also presented as ranks: 1 no bacteria or sterile; 2 denotes $<10^3$; 3 denotes 10^3; 4 denotes 10^4; 5 denotes 10^5; 6 denotes $>10^5$; 7 denotes 10^6; and 8 denotes $>10^6$ bacteria. Comorbidities were analyzed as recorded in medical history. All coronary artery diseases and heart rhythm disorders were considered heart diseases; hypertension and peripheral vascular diseases were included in the category of vascular diseases. We considered a patient to have a neurological disease if it was previously confirmed and listed in medical documentation, or if the patient had acute conditions accompanied by altered consciousness, such as bleeding in the central nervous system (CNS), trauma or ischemic stroke. Diabetes, hyperlipidemia, obesity, and endocrine disorders were considered as the same category. Laboratory values of hemoglobin, C-reactive protein (CRP), procalcitonin (PCT), and white blood cells (WBC) were recorded at the time of microbiological sampling.

Statistical analysis was performed using MS Excel and IBM Statistical Package for Social Sciences (SPSS) 22.0 statistical software (IBM SPSS Statistics; Armonk, NY, USA). Mann-Whitney test for continuous, and χ^2-test or Fisher exact test for categorical variables was calculated. A relationship between demographic parameters, comorbidities, microbiological samples and outcomes was calculated using the Spearman correlation. The level of statistical significance was set at $p<0.05$.

Results

A total of 442 bacterial isolates were obtained from 198 patients in Period A and 277 tracheal aspirates from 147 patients in Period B. Demographic data of the patients are shown in Table 1. Demographic characteristics and distribution of patients did not differ between Periods A and B. Differences were observed in the higher prevalence of metabolic and endocrine diseases in Period B, as well as in the higher prevalence of coagulopathies. Patients treated in Period B had lower hemoglobin and WBC values compared to patients treated in Period A (Table 1).

A total of 405 tracheal aspirates/29 bronchial aspirates/8 upper airway aspirates were analyzed in Period

Table 1. Demographic data of patients admitted to surgical ICU during Period A (pre-COVID-19, 2018) and Period B (post-COVID-19, 2021) with tracheal aspirates obtained

Patient characteristic	Period A (pre-COVID-19, N=198)	Period B (post-COVID-19, N=147)	p*
Age (yrs)	69 (57-78)	68 (56-77)	0.406
Sex (male/female)	125/73	90/57	0.673
ICU days	7 (4-15.75)	8 (4-15.75)	0.568
Comorbidity:			
Cardiac	79 (40)	53 (36.1)	0.445
Vascular	134 (67.7)	94 (63,9)	0.469
Respiratory	25 (12.6)	22 (14.9)	0.530
Coagulopathies	4 (2)	10 (6.8)	**0.029**
Trauma/polytrauma	43 (21.7)	36 (24.5)	0.544
Neurological	107 (54.4)	70 (47.6)	0.238
Renal	24 (12.1)	26 (17.7)	0.151
Gastrointestinal	50 (25.3)	40 (27.2)	0.682
Hepatobiliary	28 (14.1)	17 (11.6)	0.482
Metabolic/endocrine	57(28.8)	60 (40.8)	**0.02**
Sepsis on admission	47 (23.7)	48 (32.6)	0.067
Psychiatric	39 (19.7)	37 (25.1)	0.225
Neoplastic disease	51 (25.8)	39 (26.5)	0.871
Surgical/medical	161/37	34/112	0.297
Re-operated (%)	52 (25.2)	32 (21.7)	0.261
Laboratory*			
Hemoglobin (g/L)	104 (95-116)	99 (92-108)	**0.010**
WBC in blood ($x10^9$/L)	12.9 (9.5-18.3)	11.1 (7.5-16.05)	**0.001**
CRP (mg/L)	156 (102.5-254.5)	190.8 (118.45-262.75)	0.186
PCT (ug/L)	3.06 (0.97-10.7)	1.83 (0.82-11.26)	0.858

*Mann Whitney U test was used for parametric and χ^2-test or Fisher exact test for nominal data. Laboratory data were recorded on the day of the first microbiological sampling; ICU = intensive care unit; WBC = white blood cell count; CRP = C-reactive protein; PCT = procalcitonin

A and 264/9/4 samples in Period B (p=0.141). Data on bacterial isolates are shown in Table 2. Among the *Enterobacterales*, a prominent increase in the *Klebsiella* spp. isolation was confirmed. It was identified in 5 (2.5%) isolates in Period A and in 12 (13.5%) isolates in Period B (p<0.001).

Correlation analysis showed positive correlation of PMNs in tracheal aspirates with nonsterile samples (ρ=0.275, p<0.001) and type of bacteria (ρ=0.264, p>0.001) (Fig. 1), and was higher in Period A (ρ=0.164, p<0.001). A significant negative correlation was found for CRP and hemoglobin (ρ=-0.140, p<0.001), WBC (ρ=-0.123, p=0.001), and type of bacteria and year (ρ=-0.108, p=0.004). The type of bacteria differed according to the length of ICU stay until sampling (ρ=-0.159, p<0.001).

The PMN count in specific samples was associated with the type of bacteria isolated. The highest number of PMN was observed in the samples with *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* confirmed, and lowest was in sterile samples (Fig. 1).

The number of PMNs in the samples from patients with respiratory diseases was significantly higher than in patients without respiratory diseases (ρ=0.019, p=0.001), as illustrated in Figure 2.

The number of bacteria in the aspirate and the total number of non-sterile samples were associated with the length of treatment in the ICU (p<0.001) (Fig. 3). The presence of cardiac disease (ρ=0.130; p=0.016), vascular disease (ρ=0.176; p=0.001), sepsis (ρ=0.312; p<0.001) and high CRP (ρ=0.206, p<0.001) correlated

Table 2. Microbial isolates in patients admitted to surgical ICU during pre-COVID (2018, Period A) and post-COVID-19 (2021, Period B) periods

	Period A (pre-COVID-19, N=198)	Period B (post-COVID-19, N=147)	p
Total number of ICU patients (% of aspirates obtained)	790 (25)	572 (25.6)	0.789
Number of patients/tracheal aspirates sampled (samples *per* patient)	198/442 (2.23)	147/277 (1.88)	0.203
Sterile:positive (% of positive)	243/199 (45)	188/89 (32.1)	**<0.001**
PMN in tracheal samples	>25 [(10-15)-(>25)]	10-25 [(<10)-(>25)]	**<0.001**
Pathogenic microorganisms*:			
Candida spp. *(albicans, non-albicans)*	17 (3.8)	1(1.1)	**0.003**
Acinetobacter baumannii	86 (43.2)	32 (21.7)	0.247
Enterobacterales	39 (16.6)	29 (32.6)	**0.002**
Staphylococcus aureus, MRSA	23 (11.6)	15 (16.8)	0.22
MRCNS	1 (0.5)	0	1
Pseudomonas aeruginosa	29 (14.6)	7 (3)	0.112
Streptococcus β*-hemolytic*	1 (0.5)	0	1
Streptococcus pneumoniae	3 (1.5)	0	0.555
Stenotrophomonas maltophilia	0	2 (2.2)	0.095
Survival of positive patients	59%	62%	0.621
Mortality of all patients	16.8%	9.6%	<0.001

*Samples positive for bacteria in each group are presented as a total number and percentage in relation to all positive samples in a particular year. Mann Whitney U test was used for parametric and χ^2-test or Fisher exact test for nominal data. In this analysis, *Enterobacterales* comprised a diverse group of *Escherichia, Enterobacter, Citrobacter, Proteus, Serratia, Morganella, Providentia* and *Klebsiella* species that were obtained in the samples. ICU = intensive care unit; PMN = polymorphonuclear neutrophils; MRSA = methicillin resistant *Staphylococcus aureus;* MRCNS = methicillin resistant coagulase negative *Staphylococcus aureus*

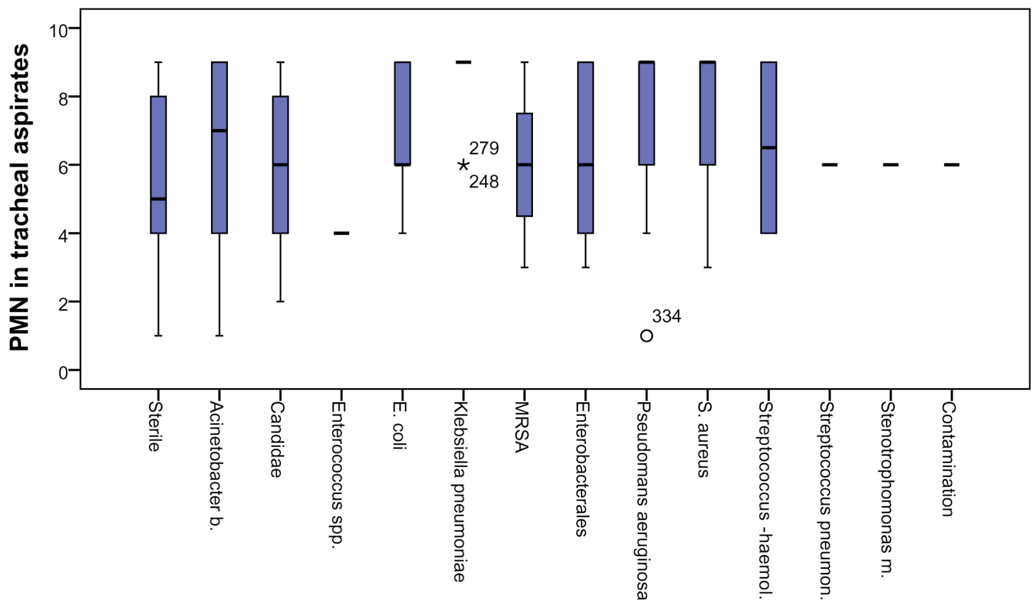

Fig. 1. Type of bacteria and polymorphonuclear neutrophils (PMN) in tracheal samples presented in ranks (Y-axis): 1 no PMN was found; 2 denotes <5; 3 denotes 5-10; 4 denotes <10; 5 denotes 10-15; 6 denotes 10-25; 7 denotes 15-20; 8 denotes 20-25; and 9 denotes >25 PMNs in the field. Escherichia coli, Klebsiella spp. and Enterococcus spp. are shown separately from other Enterobacterales.

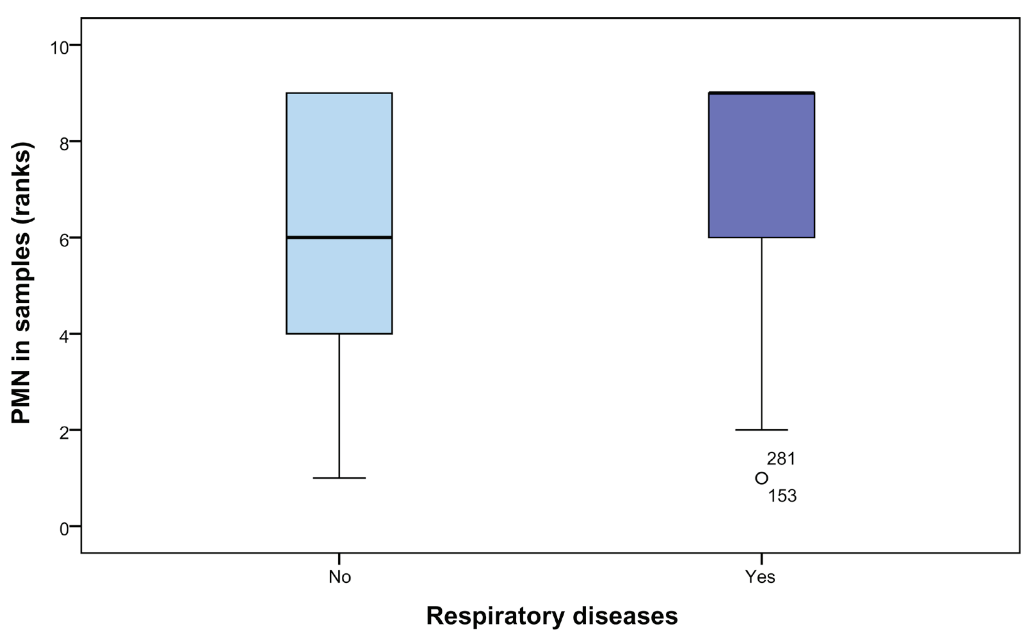

Fig. 2. Polymorphonuclear neutrophils (PMN) in tracheal aspirate samples from patients without respiratory diseases and in patients with chronic respiratory diseases. PMNs are presented in ranks (Y-axis): 1 no PMN was found; 2 denotes <5; 3 denotes 5-10; 4 denotes <10; 5 denotes 10-15; 6 denotes 10-25; 7 denotes 15-20; 8 denotes 20-25; and 9 denotes >25 PMNs in the field.

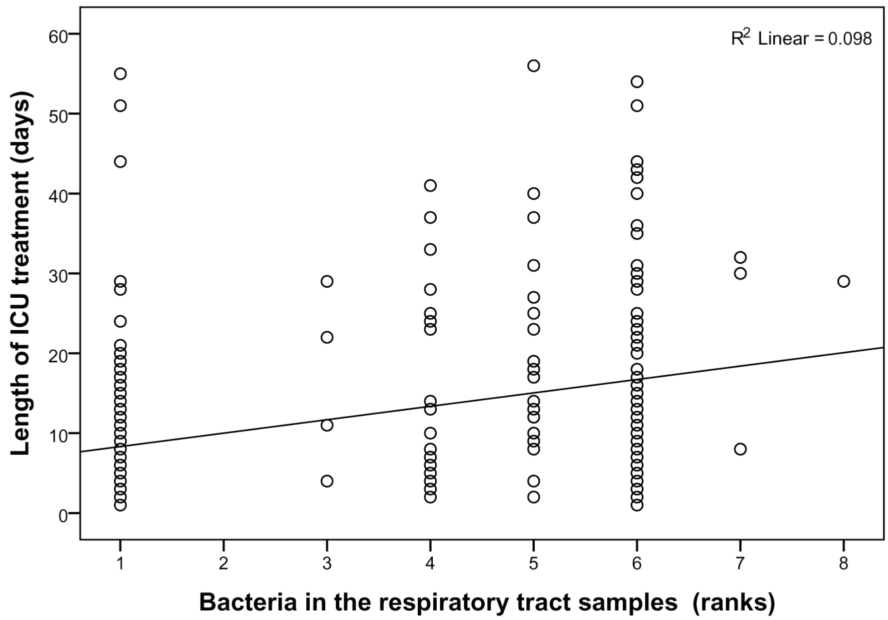

Fig. 3. Correlation between the number of bacteria in tracheal aspirates and length of intensive care unit treatment; ICU = intensive care unit. Number of bacteria (ranks): 1 no bacteria or sterile; 2 denotes <10^3 bacteria; 3 denotes 10^3; 4 denotes 10^4; 5 is for 10^5; 6 is for >10^5; 7 is for 10^6; and 8 for >10^6 bacteria.

with patient survival, but not with the type of bacteria isolated or the number of PMNs in aspirates.

Discussion

In this retrospective study analyzing a total of 719 tracheal aspirate samples, a lower prevalence of non-sterile aspirates and a lower number of PMNs in tracheal aspirates were recorded after the COVID-19 pandemic (Period B). The reason for this may be the increased awareness of medical staff about hygiene, which was also confirmed by a study from Kuwait, where after hand hygiene education, the incidence of health care-associated infections, bloodstream infections, and lower respiratory tract infections dropped significantly. Furthermore, that study confirmed a reduction of the isolation of some hospital pathogens[5].

The higher incidence of sterile samples can be linked with earlier and up-to-date laboratory diagnostics, with more accurate microbiological detection methods, and finally better targeted antibiotic therapy. After the start of the COVID-19 pandemic (Period B), MALDI-TOF was introduced in the Osijek University Hospital Center, which greatly facilitated detection and treatment of infections[4]. A Turkish study from 2020 hypothesized that MALDI-TOF provided the possibility of rapid, accessible, and inexpensive analysis of microbiological samples. It was proven that the accuracy of the method was 98.9% when diagnosing *Acinetobacter baumannii* infection or colonization. It can be assumed that MALDI-TOF will be the method of choice in bacterial identification, as well as antibiotic targeted therapy in the future[6].

In our study, the number of PMNs in tracheal aspirate was also lower in the post-pandemic period (Period B). The reason for this could be related to the previous finding that the number of PMNs in the aspirate correlates with the non-sterile finding of tracheal aspirate. Given that in Period B, a lower incidence of non-sterile aspirates was recorded, a lower number of PMNs in the aspirates could be expected. In a study by Willson *et al.*, diagnostic criteria for hospital-acquired pneumonia were, among others, more than 25 PMNs *per* low-power field on Gram stain. In their study, the number of PMNs correlated with positive bacterial culture, as observed in our results[7].

In our study, we compared the age, gender, and comorbidities of patients in relation to their stay in the ICU before and after the pandemic (Periods A and B). There were no major demographic differences between patient groups, but in Period B, fewer patients were admitted in the ICU. The reason for this may be due to postponement of elective surgical program, reassignment of patients to nonsurgical ICUs (neurological, internal medicine), along with many critically ill patients who had positive SARS CoV-2 PCR tests having been admitted and treated in the COVID ICU.

We found a difference in comorbidities, where the incidence of coagulopathy and endocrine-metabolic diseases increased in the ICU patients in Period B. Some studies have proven that the prevalence of coagulopathy in COVID-19 patients is higher among patients who stayed in the ICU[8]. In a study carried by Leentjens *et al*. that analyzed patients who had recovered from COVID-19 and who no longer required to be hospitalized, the authors suggest that routine antithrombotic prophylaxis is not recommended except for patients who have extremely high inflammatory parameters and who are immobile[9]. In our surgical ICU, patients were regularly administered low molecular weight heparin thromboprophylaxis, and the coagulopathies observed were mostly the result of using oral anticoagulants in patients with atrial fibrillation.

In this study, we analyzed endocrinological and metabolic comorbidities together. This group included diabetes, obesity, hyperlipidemia, and some other disorders. Our study confirmed that the prevalence of these diseases was higher in Period B. The sequels of COVID-19 infection may have affected the endocrine system of individuals who recovered from the disease. This also applies to the average population of ICU patients, most of whom had recovered from COVID-19 infection before being admitted to the ICU. Some studies analyzed the effect of the SARS CoV-2 virus on the endocrine system, and it has been proven that endocrine glands can be affected by the virus because they express the ACE2 receptor[10]. More prospective studies are needed to prove the long-term effects of COVID-19 on the endocrine system.

The patients who stayed in our ICU after the start of the COVID-19 pandemic (Period B) had lower levels of hemoglobin and WBCs in the blood than in Period A. This was not unexpected, as anemia is common in ICU patients. A study carried by Corvin *et al*. showed that over 95% of critically ill patients were anemic after 3 days of ICU treatment[11]. In our patients, due to delay of elective surgery program and waiting for the results of COVID-19 polymerase chain reaction tests, there is a possibility that patients were transferred to the ICU after surgery in a slightly worse general condition. Therefore, they stayed in the ICU longer, needed more laboratory tests, and may have greater probability of developing anemia. In some studies, anemia was also associated with the likelihood of complications, such as kidney injury[12].

The WBC count in the blood, which was lower in Period B, can be explained by earlier diagnosis of infection, better hand hygiene, precise targeted antibiotic therapy provided by the MALDI-TOF method[4], as well as earlier findings of a smaller number of non-sterile aspirates in this study.

Confirmation of *Candida albicans* in multiple samples of tracheal aspirates, wound swabs or urine cultures commonly observed after prolonged antibiotic therapy is a key element for starting antifungal therapy[14]. In our study, there was a lower incidence of *Candida albicans* infection in Period B. This observation can be related to the lower patient flow through ICU, and less antibiotics used. More rapid and accurate MALDI-TOF diagnostics may help reduce the time of antibiotic exposure[13]. It is expected that by reducing the total number of infections, the prevalence of *Candida* spp. isolation will also decrease, and thus the need for their treatment.

Acinetobacter baumannii was the predominant pathogen in Period A. *Acinetobacter baumannii* infections are a special challenge for ICU staff because they are particularly resistant to large spectrum of antibiotics and rapidly develop new resistance[15]. Some of the measures to prevent the spread of nosocomial *Acinetobacter* infections are staff education, hand hygiene, disposable equipment, cohorting and isolation of colonized patients[16]. Owing to these measures, the overall care of infective and ICU patients probably improved in the post-pandemic period. In an Iranian study[17] that analyzed sputum or tracheal aspirate from patients admitted to the ICU without signs of pneumonia and presumed to have developed nosocomial pneumonia, the most common pathogen was *Acinetobacter baumannii* (34.5%). In our study, that percentage was 43.4% before the pandemic and declined to 32.5%.

Of particular concern is the increase in the incidence of *Klebsiella pneumonia*e infection in our study, given that it represents a major threat to the health system as one of the most important nosocomial pathogens[18]. The same trend as confirmed in our study was observed all over the world. In the study by Sleiman *et al.*[19], extensively-drug resistant (XDR) and pandrug resistant (PDR) *Klebsiella pneumonia*e was isolated from the same patient. Considering the existence of several strains and special virulence of bacteria, a life-threatening invasive and untreatable *Klebsiella* infections might be expected in the future.

In our study, a difference in the number of PMNs in tracheal aspirate was confirmed depending on the pathogen isolated. The highest number of PMNs in the aspirate was confirmed in patients who had isolated *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* strains. This may be related to the severity of infection caused by these bacteria and the amount of microscopic purulence. This can be a special problem because a large amount of secretion clogs the airways, requires bronchoalveolar washing, thus possibly spreading the infection. Kreider and Lipson presented a case of a young man admitted to the ICU after a traffic accident. They report on a successful use of regular bronchoscopy due to airway obstruction, prevention of atelectasis, with favorable patient treatment outcome[20]. In children, the number of PMNs in tracheal aspirates is regularly monitored in pediatric ICU, and microscopic purulence is used to predict antibiotic use, as reported by Yalamanchi *et al.*; in their study, microscopic purulence was not associated with clinical symptoms[21].

The number of PMNs in the aspirate and its correlation to the type of bacteria has to be proven in future studies, since it can be a good predictor of the clinical course of nosocomial pneumonia based on secretions, microscopic purulence, and formation of atelectasis. After the COVID-19 pandemic, the ICU continues to face multi-resistant bacteria such as *Klebsiella pneumoniae*, and will continue to pose a challenge despite modern diagnostic techniques, antibiotic therapy and, finally, hygiene as the most important method of prevention.

The weak point of this study was its retrospective character. Because of this, it is possible that some data were not equally recorded. The COVID-19 pandemic has strongly confirmed the importance of comorbidities for patient outcomes. Therefore, it is possible that comorbidity was recorded more carefully after the pandemic. Due to the lower flow of patients, it is also possible that data were recorded in more detail in Period B.

The numerical value of PMNs as represented in microbiological findings may depend on various factors, among others the quality of the sample or sampling procedure, but also on the underlying disease of the patient, as well as on the laboratory staff. Therefore, a prospective, controlled study that would consider proper measurement of PMN count, possible dilution and other procedures that can affect it would probably give more reliable results on the ratio of bacteria and PMN in samples.

To conclude, there were no major demographic differences between the groups of patients who stayed in surgical ICU of the Osijek University Hospital in Periods A and B. In Period B, there was a higher prevalence of coagulopathies, metabolic and endocrine diseases in patients. The prevalence of *Candida albicans* and *Acinetobacter baumannii* isolates in tracheal aspirates was lower in Period B. A significant increase of *Klebsiella pneumoniae* isolation was recorded in Period B. The isolation *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* strains was associated with the highest number of WBC in the aspirates. There was a lower prevalence of pathogenic bacteria isolated with a lower number of PMNs in post-pandemic tracheal aspirates. This may be due to improvement in the methods of patient care.

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Sažetak

USPOREDBA TRAHEALNIH ASPIRATA U RAZDOBLJU PRIJE I NAKON POČETKA PANDEMIJE COVID-19 U JEDINICI INTENZIVNOG LIJEČENJA U TERCIJARNOJ BOLNICI

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Promjene u metodama rada i dijagnostici primjenom dijagnostike laserske desorpcije/ionizacije masene spektrometrije uz pomoć matrice (MALDI-TOF), koje su se dogodile nakon početka pandemije COVID-19 mogle bi pokazati razlike u učestalosti pozitivnih mikrobioloških uzoraka. U retrospektivnoj studiji analizirano je ukupno 442 aspirata traheje u razdoblju prije pandemije (Razdoblje A) (2018., 198 bolesnika, medijan dobi 69 (57-78) godina) i 277 uzoraka (2021., 147 bolesnika, 68 (56-77) godina, p=0,585) uzorkovanih nakon početka pandemije (Razdoblje B). Ukupno 176 bolesnika imalo je barem jedan pozitivan nalaz. U Razdoblju A bilo je 245 (55%) sterilnih uzoraka, dok ih je u Razdoblju B bilo 186 (68%) (p=0,001). Najčešće izolirani uzročnici bili su *Acinetobacter baumannii* u 86 bolesnika iz Razdoblja A i 32 iz Razdoblja B, tj. 43% naspram 21.7% svih pozitivnih izolata (p=0,247), potom *Pseudomonas aeruginosa* u 29 bolesnika u Razdoblju A (14,6%) naspram 7 (3%) (p=0,112) u Razdoblju B. Statistički značajan porast primijećen je u incidenciji *Enterobacterales* (16,6% prema 32,6%, p=0,002), osobito vrste *Klebsiellae*. Iako se ukupna smrtnost smanjila u poslijepandemijskom razdoblju (Razdoblje B), promjene u radnim metodama i dijagnostici nisu rezultirale promjenama u smrtnosti bolesnika kojima su uzorkovani aspirati dušnika.

Ključne riječi: *MALDI-TOF masena spektometrija; Pneumonija; Jedinica intenzivnog liječenja; Mikrobiologija*