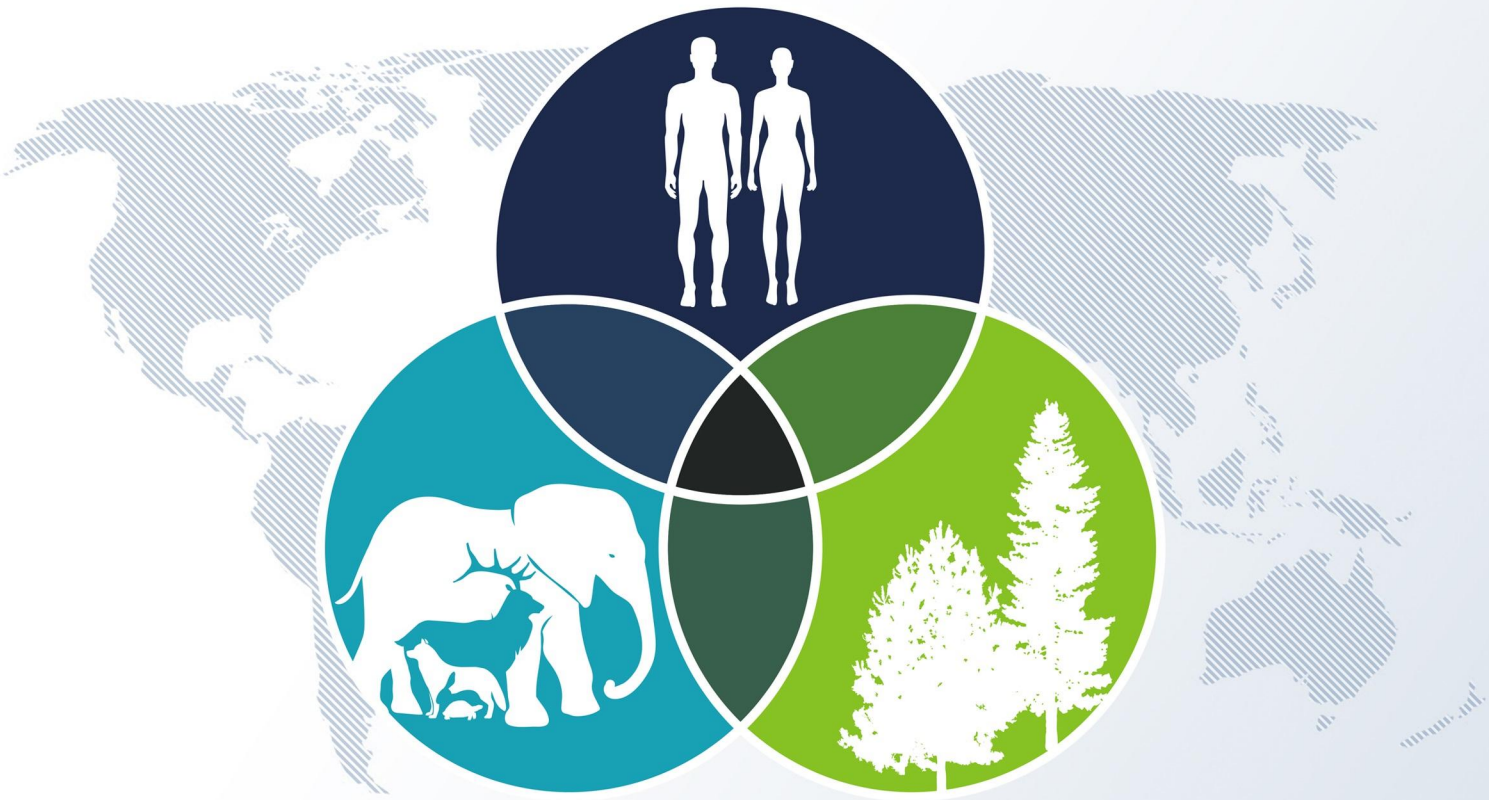
OPEN ACCESS

ISSN 2587-3458
e-ISSN 2587-3466

Category B

OH&RM ONE HEALTH & RISK MANAGEMENT

THE SCIENTIFIC JOURNAL OF THE
MOLDAVIAN BIOSAFETY AND BIOSECURITY ASSOCIATION



VOLUME 4, ISSUI 4/OCTOBER 2023

The Moldovan Association for Biosafety and Biosecurity (MDBBA) is a scientific and practical, instructive and educational, non-governmental, apolitical and non-profit professional organization, created in 2017.

The main objective of the association is the development of good practices and culture in the field of biosafety and biosecurity and the promotion of knowledge within professional and research-innovation groups.

Biosafety – includes security principles, technologies and rules to be followed to prevent unintended exposure to pathogens and toxins or their accidental release/leakage.

"Protection of personnel, population from unintended exposure to pathogens/biohazardous material".

Biosecurity - includes a wide spectrum of measures (biosecurity policies, regulatory regime, scientific and technical measures) applied in an organized framework, necessary to minimize risks (prevention of actions, terrorist attacks by the intentional release of pathogens or toxins as well as loss, their theft or misuse).

"Protection and prevention of theft, intentional misuse of pathologies/biohazardous material".

Risk management – is a decision-making process in which the results of risk assessment (the process of estimating workplace hazards) are integrated with economic, technical, social and political principles to generate strategies for risk reduction.

CONTENTS –TABLE DES MATIÈRES

Quarterly edition
Languages of publication: English, French
Founder: Asociaţia de Biosiguranţă şi Biosecuritate din Republica Moldova

Category B

ISSN 2587-3458 (Print)
e-ISSN 2587-3466 (Online)
Edited by: Typography "Print-Caro", Edition: 300 ex.
Registered at the Ministry of Justice with no. 476676, 05th of July, 2017

Invasive fungal infections in healthcare settings

Maria BALASOIU, PhD
Laborator of Microbiology, University of Medicine and Pharmacy of Craiova,
Head of Medical Analysis Laboratory,
County Emergency Clinical Hospital,
Craiova, Romania

The incidence of invasive infections produced by species of Candida genus is continuously raising, Candida is the 5th most common pathogen in invasive infections. Although most of the fungal invasive infections are produced by Candida albicans, there are increasing reports of other non-albicans species that produce severe infections because have increased pathogenicity and resistance to commonly used fungal agents, with high mortality rate. Non-albicans Candida species identified in recent years include C. glabrata, C. parapsilosis, C. tropicalis and C. auris, the last seems to have an uniquely high transmissibility in hospital environments and also between healthcare settings.

C. auris outbreaks are systematically reported in recent years, and molecular investigation had confirmed the intra-hospital transmission. The first reported invasive infection with C. auris was in South Korea in 1996, since then outbreaks had been reported in more than 40 countries on 6 continents.

Recently, Candida isolates have demonstrated increased resistance to azoles, amphotericin B and echinocandins, many strains are multidrugresistant or even pan-resistant.

The national health services must enforce preventive measures of invasive fungal infections, especially in high-risk patient groups as those with impaired immune system (anticancer drugs, long-term corticoid treatments, solid organ transplant and other chronic diseases). The laboratories must be prepared to identify this emerging fungal pathogens by at least biochemical methods, but it is reccomended to use molecular methods as PCR, MLST, and MALDI-TOF MS. Screening protocols must be implemented in all patients at risk.

OH&RM ONE HEALTH & RISK MANAGEMENT

VOL. 4, ISSUE 4
2023

SYNTHESIS ARTICLE – ARTICLES DE SYNTHÈSE

MEDICAL SCIENCES

OPEN ACCESS

PROGNOSTIC VALUE OF D-DIMERS IN PATIENTS WITH COVID-19: NARRATIVE SYNTHESIS

Natalia CERNEI[1,2], Ruslan BALTAGA[1,3], Serghei SANDRU[1,2], Olga GHERASIM[1,2], Victoria MOGHILDEA[1,2]

[1]*Nicolae Testemitanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova
[2]Institute of Emergency Medicine, Chisinau, Republic of Moldova
[3]Institute of Oncology, Chisinau, Republic of Moldova

Corresponding author: Natalia Cernei, e-mail: nataliacernei1@gmail.com

DOI: 10.38045/ohrm.2023.4.01 CZU: [616.98:578.834.1]-074-037

Keywords: *COVID-19, SARS-CoV-2, D-dimers, venous thromboembolism, biomarkers.*

Introduction. *Contemporary researchers have suggested and demonstrated the hypothesis that the elevated level of D-dimers, which is a valuable marker of coagulation and fibrinolysis activation, can predict the severity of COVID-19, pulmonary complications, and thromboembolic events before they occur.* ***Material and methods.*** *The bibliographic resources were analyzed and selected from databases such as PubMed, Hinari, SpringerLink, and Google Search using keywords such as "COVID-19," "SARS-CoV-2," "coronavirus," "D-dimers," "biomarkers," and "severity prediction," which were used in various combinations to maximize search efficiency. Therefore, the manuscript contains 51 representative articles for the purpose of this synthesis article.* ***Results.*** *The D-dimer levels are significantly higher in patients with severe forms of COVID-19 compared to those with non-severe forms, in patients with acute respiratory distress syndrome compared to those without acute respiratory distress syndrome, and in deceased patients compared to those who have survived. D-dimers positively correlate with the degree of severity and the increased risk of progression to severe disease, inversely proportional to the survival rate. They can predict prognosis, determine therapeutic strategies, prevent complications, positively influence the disease's course, and monitor the prognosis.* ***Conclusions.*** *D-dimers should be used as a pre-radiographic screening tool as early as possible after admission and as an indicator for risk stratification of venous thromboembolism in hospitalized patients with COVID-19. Based on the increase in D-dimer levels, adjusting therapeutic doses of anticoagulants is more beneficial for patients compared to administering prophylactic doses.*

Cuvinte-cheie: *COVID-19, SARS-CoV-2, D-dimeri, tromboembolism venos, biomarkeri.*

VALOAREA PROGNOSTICĂ A D-DIMERILOR LA PACIENȚII CU COVID-19: SINTEZĂ NARATIVĂ

Introducere. *Cercetătorii contemporani au sugerat și demonstrat ipoteza că nivelul crescut al D-dimerilor, care este un marker valoros de activare a coagulării și fibrinolizei, pot prezice severitatea bolii COVID-19, complicațiile pulmonare și evenimentele tromboembolice înainte ca acestea să survină.* ***Material și metode.*** *Resursele bibliografice au fost analizate și selectate din bazele de date PubMed, Hinari, SpringerLink și Google Search după cuvintele-cheie: „COVID-19", „SARS-CoV-2", „coronavirus", „D-dimeri", „biomarkeri", „predicția severității", care au fost folosite în diferite combinații pentru a maximiza randamentul căutării. Astfel, cuprinsul manuscrisului include 51 de articole reprezentative pentru scopul acestui articol de sinteză.* ***Rezultate.*** *Valoarea D-dimerilor este semnificativ mai mare la pacienții cu forme severe de COVID-19, comparativ cu cei cu forme non-severe, la pacienții cu sindrom de detresă respiratorie acută, comparativ cu cei fără sindrom de detresă respiratorie acută, la pacienții decedați, comparativ cu cei care au supraviețuit. D-dimerii corelează pozitiv cu gradul de severitate și riscul crescut de progresare la forma severă a maladiei, invers proporțional cu rata de supraviețuire, pot prezice prognosticul, determinarea strategiilor terapeutice, prevenirea complicațiilor, influențarea pozitivă a evoluției maladiei și supravegherea prognosticului.* ***Concluzii.*** *D-dimerii trebuie utilizați ca instrument de screening pre-radiografic cât mai precoce după internare și ca indicator de stratificare a riscului de tromboembolism venos pentru pacienții internați cu COVID-19. În baza creșterii D-dimerilor, ajustarea dozelor terapeutice de anticoagulante este mai benefică pentru pacienți, comparativ cu administrarea dozelor profilactice.*

INTRODUCTION

Since December 2019, a new coronavirus, called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been declared responsible for triggering the epidemic of coronavirus disease 2019 (COVID-19), which rapidly spread worldwide. Consequently, on March 11, 2020, the World Health Organization declared a global public health emergency – a state of pandemic (1, 2, 3). The clinical presentation of COVID-19 varies from asymptomatic and mild forms to severe and critical cases that progress rapidly, causing serious and fatal complications requiring admission to the intensive care unit (ICU) (1, 3, 4-9). The overall mortality rates for patients with COVID-19 range from 1.5% to 9.8% (10, 11), while mortality rates for patients admitted to the ICU range from 26% to 61.5% (4, 6, 9, 12).

One of the strategic elements that need to be undertaken in defining the management of treatment for patients with COVID-19 is the early identification of factors that may progress to more severe forms of the disease and those that require specific interventions or treatments (13). Based on the pathophysiology of severe COVID-19, which involves a hyperinflammatory state, a cascade of hypercoagulation, and multiorgan dysfunction, several biomarkers that represent each of these conditions could be useful as key predictive values for COVID-19. In this context, defining early and efficient predictors for patient outcome prognosis holds substantial clinical significance for risk stratification, severity prediction, management of patients with COVID-19, and prevention of serious complications. In emergency situations, risk stratification is as important as diagnosis, especially if testing all patients suspected of having COVID-19 is not possible (1, 8, 10, 14-20).

COVID-19 causes serious thromboembolic complications in patients with severe forms of the disease (7, 21). COVID-19-related mortality is largely associated with hypercoagulability and an increased risk of venous thromboembolism (VTE), leading to thrombo-inflammation in severe cases. Therefore, coagulation biomarkers can indicate disease severity, mortality, and contribute to patient triage, therapeutic strategies, and close monitoring of prognosis (5, 8, 21-27).

Excessive inflammation, platelet activation, endothelial dysfunction, and stasis play a significant role in the development of thrombotic complications (10, 17). Coagulation abnormalities such as hypercoagulability, thrombocytopenia, venous thrombosis, and disseminated intravascular coagulation have been observed in approximately 60-70% of hospitalized patients with COVID-19. Autopsies have shown that pulmonary embolism or venous thrombosis was the cause of death in about 58% of patients, while disseminated intravascular coagulation was reported in 70% of patients (28).

Researchers have suggested that the increased level of D-dimers (DD), which is a valuable marker of coagulation and fibrinolysis activation, can predict disease severity, pulmonary complications, and thromboembolic events before they occur (29-32). DD are degradation products of reticulated fibrin, a substance that is part of fibrin clots. Its presence itself implies venous thromboembolism (pulmonary embolism and deep vein thrombosis). DD are a marker of active coagulation, fibrinolytic events, and thrombotic activity, with elevated values in patients with vascular thrombosis caused by severe trauma, inflammatory and infectious conditions, pregnancy, inflammation, cancer, etc. Several studies have highlighted the correlation between elevated DD levels, especially their gradual increase during the course of the disease, with the severity and adverse outcomes of COVID-19. DD levels correlate with the severity of COVID-19, thrombotic activity, and mortality rates in these patients (2, 3, 16, 22, 27, 29, 31, 32, 33). The DD level significantly increases concomitantly with the increasing severity of clinical manifestations and with the findings of computed tomography investigations of COVID-19 (19, 32, 34).

Thus, elevated levels of D-dimers reflect significant activation of the coagulation cascade, which, in turn, can trigger acute coronary syndrome and pulmonary embolism with its full spectrum of clinical manifestations (1). However, the prognostic value of D-dimers, determined upon admission, in predicting disease severity, mortality, and thromboembolic events in COVID-19, has not been fully elucidated due to small cohorts and heterogeneity among studies (14, 17, 22, 27, 30, 35, 36).

In the context of the aforementioned points and considering that the clinical and laboratory characteristics associated with SARS-CoV-2 infection

are not fully elucidated (37), the *aim* of this narrative synthesis is to summarize the analysis and clinical significance of elevated D-dimer levels, their prognostic role, and their association with severity, adverse outcomes, thromboembolic events, and mortality in patients with COVID-19.

MATERIAL AND METHODS

The bibliographic resources were analyzed and selected from databases such as *PubMed, Hinari, SpringerLink*, and *Google Search* using keywords: "COVID-19," "SARS-CoV-2," "coronavirus," "D-dimers," "biomarkers," and "severity prediction"; which were used in various combinations to maximize search efficiency.

For targeted selection of bibliographic sources, the following filters were applied: full-text articles, articles in the English language, articles published between 2020-2022. After processing the identified information and according to the search criteria, 386 full-text articles were selected. After excluding records unrelated to the study's aim and reviewing the abstracts and full texts of the articles, 45 eligible original papers with different study designs, including editorials, narrative and systematic review articles, meta-analyses, case-control studies, and cohort studies, containing information about D-dimers in patients with COVID-19, were considered potentially relevant for the given synthesis.

According to the proposed aim and after careful evaluation and analysis of these sources, a total of 51 relevant publications were ultimately selected and included in the final bibliography of this manuscript. These publications were deemed representative of the materials published on the subject of this synthesis article.

The information from the publications included in the bibliography was gathered, classified, evaluated, and synthesized, highlighting the main aspects of the contemporary view regarding the role of D-dimers in patients with COVID-19, the correlation and prediction of clinical outcomes, severity, and prognosis of SARS-CoV-2 infection.

A primary objective was to minimize the risk of systematic errors (bias) in the study by conducting thorough searches in databases to identify a maximum number of relevant publications for the study's purpose. We evaluated only those studies that met valid criteria and applied strict exclusion criteria for article selection. We assessed both studies reporting optimistic data and research that did not highlight the benefit of D-dimer determinations in patients with COVID-19.

According to the requirements, additional sources of information were accessed to clarify specific and sophisticated concepts. Similar research, articles that did not align with the purpose of the study and were not available for full viewing, review articles, comments, and letters, case reports or case series, articles with insufficient information, articles lacking data on D-dimer concentrations, non-human studies, and studies on pediatric populations (<17 years) were excluded from the bibliography.

RESULTS

Coagulation dysfunction in patients with COVID-19 leads to the progression to severe form of the disease and fatal outcomes, and it is characterized by increased D-dimer levels and thromboembolic events (5, 22, 23). Several studies have assessed the association between initial D-dimer values and the severity or outcomes in patients with COVID-19.

A systematic review, conducted on 54 studies involving 1,022 COVID-19 patients, and two retrospective studies, including 343 and 182 patients respectively, with 13 and 34 in-hospital death cases, found a significantly higher mean D-dimer value at admission ($p<0.001$) in deceased patients (3.208-3.78 μg/mL) compared to survivors (0.79-1.067 μg/mL) (14, 32, 38). The authors calculated the optimal cutoff value determined at admission to predict in-hospital mortality – 1.5 μg/mL, with a sensitivity of 70.6% and specificity of 78.4%, and 2.0 μg/mL, with a sensitivity of 92.3% and specificity of 83.3% (14, 38).

A case-control study conducted on 248 COVID-19 patients found a significantly higher level of D-dimer in non-survivors compared to survivors (6.21 mg/L and 1.02 mg/L, $p<0.05$). D-dimer level >2.0 mg/L at admission was the only variable associated with increased odds of mortality (OR 10.17; $p<0.05$). D-dimer elevation (≥0.5 mg/L) was observed in 74.6% of patients. D-dimer level >2.14 mg/L predicted in-hospital mortality with a sensitivity of 88.2% and specificity of 71.3% (34).

A retrospective study of a cohort of 483 COVID-19 patients found that D-dimer elevation

(≥0.5 mg/mL) was observed in 80.1% of hospitalized patients and in 96% of cases that resulted in death. D-dimer level ≥2.01 mg/mL, with a sensitivity of 73.3% and specificity of 70.0%, was a significant predictor of subsequent mortality (HR 3.165; p<0.01). The median value of D-dimer among non-survivors was 6.34 mg/mL, while among survivors it was 0.94 mg/mL. Thus, a D-dimer value ≥2.01 mg/mL can effectively predict in-hospital mortality in patients with COVID-19 (39), and monitoring D-dimer during hospitalization is a better predictor of disease progression, severity, and mortality compared to D-dimer levels at admission (5, 39).

Numerous retrospective, prospective, cohort, and case-control studies, systematic reviews, and meta-analyses have reported an increased D-dimer level in a significant positive relationship with disease severity, composite outcome (including death, severe disease, ICU admission, and mechanical ventilation), VTE incidence, and mortality in patients with COVID-19 (1, 12, 15, 16, 21, 22, 24, 25, 26, 31, 34, 35, 40, 41). Elevated D-dimer levels within the first week of hospitalization in patients with COVID-19 have been associated with increased mortality and VTE incidence (20, 36, 40). The time for VTE screening in a patient is when the D-dimer level is three times higher than the upper limit of normal (0.5 μg/mL). In COVID-19 patients, a four-fold increase in the D-dimer level is a good predictor of mortality (32).

Uncontrolled inflammation combined with hypoxia and the direct cytotoxic effects of the virus on endothelial cells contribute to thromboembolic complications (12, 38, 42). Coagulation abnormalities are common in all severe infections and inflammations, but they occur more frequently and with a clinically severe prognosis in patients infected with SARS-CoV-2 (23).

SARS-CoV-2 typically induces a significant thrombo-inflammatory response in severe forms of COVID-19 with progressive diffuse pulmonary involvement (33). The most commonly cited reason in the literature for the increase in D-dimer levels includes viremia and the cytokine storm syndrome, with a significant rise in proinflammatory cytokines and inadequate control of anti-inflammatory factors, which dysregulate the coagulation cascade (38).

Mortality related to COVID-19, a prothrombotic condition, is largely associated with hypercoagulability and an increased risk of VTE, leading to thromboinflammation in severe cases (22, 23). For instance, according to data from a recent meta-analysis, the incidence of pulmonary embolism was estimated at 8% in the general population of patients with COVID-19 and at 17% in ICU-admitted patients (23). Coagulopathy occurs in 50% of patients who die from COVID-19 (42).

Therefore, coagulation biomarkers, including D-dimers, are positively associated with the severity of the disease and inversely proportional to survival. They can predict the prognosis and outcome of patients with COVID-19, including mortality. They contribute to patient triage, determination of therapeutic strategies, prevention of complications, positively influencing disease progression, and monitoring of prognosis (1, 2, 3, 22, 23, 28, 31, 34, 38).

D-dimer levels are higher in hospitalized patients with COVID-19 (36-43%) (8, 29). DD measured at admission serves as an accurate biomarker for predicting mortality in patients with COVID-19, with a specific normal value <0.5 μg/mL (21, 27, 34, 38, 43). DD level >1 μg/mL is considered a risk factor for mortality in hospitalized adult patients with COVID-19 (34). According to the results of multiple studies, a DD value at admission ≥2.38 μg/mL was associated with increased chances of mortality. Each 1 μg/mL increase in DD level at admission was associated with a 6% increase in the risk of all-cause mortality, an 8% increase in the risk of assisted ventilation, and an 8% increase in the risk of thromboembolism (44).

Therefore, elevated D-dimer levels at admission in patients with SARS-CoV-2 infection have been associated with an increased risk of escalation to severe form of the disease and death (2, 23, 28, 31, 38).

A systematic review and meta-analysis conducted on 12 studies involving 2,794 patients with COVID-19, including 596 (21.3%) patients with severe form and 2,198 (78.7%) patients with non-severe form of the disease, revealed that elevated D-dimer levels and low platelet count were more common in patients with severe condition (30). Increased DD level (OR: 5.67) and thrombocytopenia (OR: 3.61) predicted severe infection, enabling early identification and management of patients with negative outcomes. The elevated DD level and low platelet count may suggest the activation of systemic coagulation with secondary fi-

brinolysis and platelet consumption. This finding confirms the prothrombotic phenotype of SARS-CoV-2 infection, which is associated with disease severity (16, 30, 45).

The evaluation of three systematic reviews and meta-analyses conducted on 12-23 studies with a variable number of patients with COVID-19 included (7-4848) determined a significantly higher DD level in patients with COVID-19 compared to healthy individuals (2, 12, 34), in patients with severe forms of COVID-19 compared to those with non-severe forms (2, 6, 12, 18, 34), in patients with acute respiratory distress syndrome (ARDS) compared to those without ARDS, and in deceased ARDS patients compared to those who survived ($p<0.001$). Patients with COVID-19 treated with anticoagulants, due to the reversal of the procoagulant pattern, had lower mortality compared to untreated patients ($p<0.05$) (2, 12, 13, 34).

Six large-scale systematic reviews and meta-analyses, conducted on 6-75 studies with 1,329-17,052 hospitalized patients with COVID-19, revealed that an increase in DD level was associated with a 2-3 times higher risk of poor composite outcome (including mortality, severe COVID-19, ARDS, ICU admission, and mechanical ventilation), a 2-fold higher risk of developing severe COVID-19, and a 4-fold higher risk of mortality (11, 24, 35, 43, 46, 47). Subgroup analysis showed that the elevated DD level was associated with increased mortality: 21% versus 4.9% ($p<0.001$), RR 4.11-4.77 ($p<0.001$) (27, 35, 47); OR 28.14 ($p<0.001$) (13, 27, 35, 48). The elevated DD level was also associated with severe COVID-19: 40.74% versus 21.98% ($p<0.001$), RR 2.04-2.42 ($p<0.001$) (13, 35, 47, 48). The DD level was higher in the severe/non-survivor group compared to the non-severe/survivor group (2.9 and 0.8 mg/dL, respectively; $p<0.001$) (48).

The results of a systematic review and meta-analysis were based on 39 studies reporting DD levels in 5,750 non-severe patients and 2,063 severe patients, and 16 studies reporting DD levels in 2,783 survivors and 697 non-survivors. DD levels were significantly higher in patients with severe clinical status compared to those with non-severe forms of COVID-19, and in non-survivors compared to survivors. DD levels above the upper limit of normal were associated with a higher risk of severity (RR: 1.58; $p<0.0001$) and mortality (RR: 1.82; $p<0.0001$). The authors concluded that elevated DD levels determined at admission correlate significantly with the severity of COVID-19 and can predict mortality in hospitalized patients, thus they should be used for risk stratification in COVID-19 patients (17). Another systematic review and meta-analysis involving 6 original studies and a total of 1,355 hospitalized patients with moderate to critical COVID-19 confirmed these results – DD level is significantly associated with the risk of mortality in COVID-19 patients. The average DD value was higher in non-survivors compared to survivors (49).

According to the results of four meta-analyses published in 2020 and 2021, the sensitivity of the prognostic performance of DD for severity, mortality, and VTE in patients with COVID-19 was 55-77%, 64-75%, and 90% respectively, while the specificity was 56-71%, 66-83%, and 60% respectively. DD is considered a global marker of hemostasis activation and can predict severe and fatal outcomes in COVID-19 patients with moderate sensitivity and specificity. It can diagnose VTE with high sensitivity and low specificity. The authors recommend the use of this marker as a pre-radiographic screening tool, risk stratification indicator for VTE, and routine investigation after anticoagulant therapy for hospitalized patients with COVID-19 (2, 12, 22, 29).

DISCUSSIONS

DD is an independent prognostic marker. Higher DD levels in patients with COVID-19 are significantly associated with the risk of disease progression, severity of the condition, composite outcome, and mortality risk (12). These meta-analyses recommend the rapid assessment of DD for predicting adverse outcomes in COVID-19 (2, 12, 22, 29).

Patients with severe forms of COVID-19 have a higher risk of hypercoagulability, and deceased patients show significantly higher DD levels, reflecting a state of hypercoagulability. These findings suggest that higher DD levels in patients with COVID-19 may indicate coagulopathy and thrombotic risk (12, 48, 50).

Although the DD levels at admission and their trends during hospitalization are associated with outcomes in COVID-19, DD has limited performance characteristics as prognostic tests when analyzed separately (44).

A systematic review and meta-analysis conducted on 12 studies with 3,343 patients, including 2,801 patients with COVID-19 (967 patients with severe form of the disease), revealed that the pooled results of all studies showed significantly higher DD concentrations in patients with more severe COVID-19 compared to patients with non-severe condition and the overall COVID-19 patients (29). There are correlations between the severity of COVID-19, severe increase in DD levels, and the increased rate of complications and final outcomes (2).

Therefore, DD should be used as a pre-radiographic screening tool as early as possible after admission, as a risk stratification indicator for VTE, and as a routine investigation after anticoagulant therapy for hospitalized patients with COVID-19. Based on the increase in DD levels, adjusting therapeutic doses of anticoagulants becomes more beneficial for patients compared to prophylactic doses (22).

A D-dimer level >2590 ng/mL was associated with a 17-fold increase in the adjusted risk of pulmonary embolism, and the absence of any anticoagulant therapy was associated with a 4-fold increase in the risk of pulmonary embolism in patients with severe COVID-19 (oxygen saturation measured by pulse oximetry SpO_2 ≤93% in room air, respiratory rate ≥30 breaths/min, or rapid clinical deterioration) (51).

It is confirmed that severe COVID-19 is associated with cascades of inflammatory mediators, and the evaluation of these markers allows for early identification or even prediction of disease progression. It is well known that C-reactive protein is an acute-phase protein and an active regulator of innate immunity, serving as a predictive proinflammatory biomarker for the need of mechanical ventilation and escalation of treatment in uncontrolled inflammation in SARS-CoV-2 viral infection (38).

Therefore, early identification of variables associated with poor outcomes in patients with COVID-19 can be useful for planning more appropriate preventive treatment, reducing the risk of developing ARDS and ICU admission, improving survival, and optimizing the allocation of healthcare resources, which can be highly limited in some countries. Additionally, ambulatory patients presenting one or more of these characteristics can be promptly hospitalized for enhanced management (16, 30).

Furthermore, the assessment of DD concentration and its correlation with inflammatory markers and the severity of COVID-19 has found that the median value of DD was three times higher in patients with severe forms of COVID-19 compared to those with mild forms of the disease (1870 mg/L versus 630 mg/L, respectively). A weak but significant positive correlation was observed between DD and CRP ($r=0.327$; $p<0.001$). The combination of CRP value of 72.65 mg/L and DD value of 1250 mg/L can be used as a marker of COVID-19 severity with moderate accuracy (42).

Therefore, based on the aforementioned information, it is evident that COVID-19 is a prothrombotic condition, and coagulopathy is a significant complication in such patients, closely associated with clinical outcomes. DD indicates hypercoagulability and is directly associated with the severity of COVID-19. Furthermore, DD can be used as an early and reliable prognostic biomarker to predict mortality in patients with COVID-19 at the time of hospitalization. They facilitate a personalized and effective clinical management process, including anticoagulation strategies, which could significantly reduce the mortality rate (3, 12, 22, 24, 29, 34, 44, 45, 48, 49).

The determination of DD is a widely available laboratory test, feasible in patients with COVID-19 (16, 17, 24, 38, 49). However, there are also sources of heterogeneity, including age, comorbidity rates, average length of hospitalization, criteria for excluding conditions that increase DD levels (such as pregnancy, cancer, post-trauma state, and surgery), and the timing of DD measurement. Furthermore, the lack of association between DD levels and mortality indicates that anticoagulant treatment may lead to a decrease in the number of deaths (22, 45). Confirmation of these results requires further studies, particularly on a global scale, to determine the specific key value of this laboratory biomarker (6, 18).

CONCLUSIONS

1. COVID-19 is a prothrombotic, procoagulant condition, and coagulopathy is a common complication in such patients closely associated with clinical outcomes. In the majority of cases, D-dimer levels

are elevated and indicate hypercoagulability that is directly proportional to the severity of COVID-19.

2. D-dimers are positively associated with the degree of severity and increased risk of progression to a severe form of the disease. They correlate inversely with survival and can predict the prognosis and outcome of patients with COVID-19. D-dimers contribute to patient triage, determining therapeutic strategies, preventing complications, positively influencing the course of the disease, and monitoring prognosis.
3. The level of D-dimers is significantly higher in patients with COVID-19 compared to healthy individuals, in patients with severe forms of COVID-19 compared to those with non-severe forms, in patients with ARDS compared to those without ARDS, and in deceased patients compared to those who have survived.
4. D-dimers should be used as a pre-radiographic screening tool as early as possible after admission, as an indicator for stratifying the risk of venous thromboembolism, and as a routine investigation after anticoagulant therapy for hospitalized patients with COVID-19. Based on the increase in D-dimers, adjusting therapeutic doses of anticoagulants is more beneficial for patients compared to administering prophylactic doses.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

REFERENCES

1. Figliozzi S, Masci P, Ahmadi N, Tondi L, Koutli E, Aimo A et al. Predictors of adverse prognosis in COVID-19: A systematic review and meta-analysis. *Eur J Clin Invest*. 2020;50(10):e13362. doi:10.1111/eci.13362
2. Vidali S, Morosetti D, Cossu E, Luisi M, Pancani S, Semeraro V et al. D-dimer as an indicator of prognosis in SARS-CoV-2 infection: a systematic review. *ERJ Open Res*. 2020;6(2):00260-2020. doi:10.1183/23120541.00260-2020
3. Nasif W, El-Moursy Ali A, Hasan Mukhtar M, Alhuzali A, Yahya Alnashri Y, Ahmed Gadah Z et al. Elucidating the Correlation of D-Dimer Levels with COVID-19 Severity: A Scoping Review. *Anemia*. 2022; 2022:9104209. doi:10.1155/2022/9104209
4. Siddiqi HK, Mehra MR. COVID-19 illness in native and immunosuppressed states: A clinical-therapeutic staging proposal. *J Heart Lung Transplant*. 2020;39(5):405-407. doi:10.1016/j.healun.2020.03.012
5. Tjendra Y, Al Mana A, Espejo A, Akgun Y, Millan N, Gomez-Fernandez C et al. Predicting Disease Severity and Outcome in COVID-19 Patients: A Review of Multiple Biomarkers. *Arch Pathol Lab Med*. 2020;144(12):1465-1474. doi:10.5858/arpa.2020-0471-SA
6. Ghahramani S, Tabrizi R, Lankarani K, Kashani S, Rezaei S, Zeidi N et al. Laboratory features of severe vs. non-severe COVID-19 patients in Asian populations: a systematic review and meta-analysis. *Eur J Med Res.* 2020;25(1):30. doi:10.1186/s40001-020-00432-3
7. Shang W, Dong J, Ren Y, Tian M, Li W, Hu J et al. The value of clinical parameters in predicting the severity of COVID-19. *J Med Virol.* 2020;92(10): 2188-2192. doi:10.1002/jmv.26031
8. Ponti G, Maccaferri M, Ruini C, Tomasi A, Ozben T. Biomarkers associated with COVID-19 disease progression. *Crit Rev Clin Lab Sci*. 2020;57(6): 389-399. doi:10.1080/10408363.2020.1770685
9. Bastug A, Bodur H, Erdogan S, Gokcinar D, Kazancioglu S, Kosovali B et al. Clinical and laboratory features of COVID-19: Predictors of severe prognosis. *Int Immunopharmacol*. 2020;88:106950. doi:10.1016/j.intimp.2020.106950
10. Grieb P, Swiatkiewicz M, Prus K, Rejdak K. Hypoxia may be a determinative factor in COVID-19 progression. *Curr Res Pharmacol Drug Discov*. 2021;2:100030. doi:10.1016/j.crphar.2021.100030
11. Wu Y, Li H, Zhang Z, Liang W, Zhang T, Tong Z et al. Risk factors for mortality of coronavirus disease 2019 (COVID-19) patients during the early outbreak of COVID-19: a systematic review and meta-analysis. *Ann Palliat Med*. 2021;10(5):5069-5083. doi:10.21037/apm-20-2557
12. Varikasuvu S, Varshney S, Dutt N, Munikumar M, Asfahan S, Kulkarni P et al. D-dimer, disease severity, and deaths (3D-study) in patients with COVID-19: a systematic review and meta-analysis of 100 studies. *Sci Rep*. 2021;11(1):21888. doi:10.1038/s41598-021-01462-5
13. Melo AK, Milby KM, Caparroz AL, Pinto AC, Santos RR, Rocha AP et al. Biomarkers of cytokine storm as red flags for severe and fatal COVID-19 cases: A living systematic review and meta-analysis. *PLoS One*. 2021;16(6):e0253894. doi:10.1371/journal.pone.0253894

14. Zhang L, Yan X, Fan Q, Liu H, Liu X, Liu Z et al. D-dimer levels on admission to predict in-hospital mortality in patients with Covid-19. *J Thromb Haemost*. 2020;18(6):1324-1329. doi:10.1111/jth.14859
15. Gao Y, Li T, Han M, Li X, Wu D, Xu Y et al. Diagnostic utility of clinical laboratory data determinations for patients with the severe COVID-19. *J Med Virol*. 2020;92(7):791-796. doi:10.1002/jmv.25770
16. Statsenko Y, Al Zahmi F, Habuza T, Gorkom K, Zaki N. Prediction of COVID-19 severity using laboratory findings on admission: informative values, thresholds, ML model performance. *BMJ Open*. 2021;11(2):e044500. doi:10.1136/bmjopen-2020-044500
17. Gungor B, Atici A, Baycan O, Alici G, Ozturk F, Tugrul S et al. Elevated D-dimer levels on admission are associated with severity and increased risk of mortality in COVID-19: A systematic review and meta-analysis. *Am J Emerg Med*. 2021;39:173-179. doi:10.1016/j.ajem.2020.09.018
18. Hariyanto T, Japar K, Kwenandar F, Damay V, Siregar J, Lugito N et al. Inflammatory and hematologic markers as predictors of severe outcomes in COVID-19 infection: A systematic review and meta-analysis. *Am J Emerg Med*. 2021;41:110-119. doi:10.1016/j.ajem.2020.12.076
19. Taj S, Kashif A, Arzinda Fatima S, Imran S, Lone A, Ahmed Q. Role of hematological parameters in the stratification of COVID-19 disease severity. *Ann Med Surg* (Lond). 2021;62:68-72. doi:10.1016/j.amsu.2020.12.035
20. Gabbai-Armelin PR, de Oliveira AB, Ferrisse TM, Sales LS, Barbosa ER, Miranda ML et al. COVID-19 (SARS-CoV-2) infection and thrombotic conditions: A systematic review and meta-analysis. *Eur J Clin Invest*. 2021;51(6):e13559. doi:10.1111/eci.13559
21. Zheng Z, Peng F, Xu B, Zhao J, Liu H, Peng J et al. Risk factors of critical & mortal COVID-19 cases: A systematic literature review and meta-analysis. *J Infect*. 2020;81(2):e16-e25. doi:10.1016/j.jinf.2020.04.021
22. Zhan H, Chen H, Liu C, Cheng L, Yan S, Li H et al. Diagnostic Value of D-Dimer in COVID-19: A Meta-Analysis and Meta-Regression. *Clin Appl Thromb Hemost*. 2021;27:10760296211010976. doi:10.1177/10760296211010976
23. Javorac J, Živanović D, Stojkov S, Miličić J, Fradelos E, Savić N. COVID-19 associated pulmonary embolism with D-dimer values within the referent range: a case report and review of the literature. *Eur Rev Med Pharmacol Sci.* 2021;25(24):7971-7975. doi:10.26355/eurrev_202112_27647
24. Malik P, Patel U, Mehta D, Patel N, Kelkar R, Akrmah M et al. Biomarkers and outcomes of COVID-19 hospitalisations: systematic review and meta-analysis. BMJ Evid Based Med. 2021;26(3):107-108. doi:10.1136/bmjebm-2020-111536
25. Berger J, Kunichoff D, Adhikari S, Ahuja T, Amoroso N, Aphinyanaphongs Y et al. Prevalence and Outcomes of D-Dimer Elevation in Hospitalized Patients With COVID-19. *Arterioscler Thromb Vasc Biol*. 2020;40(10):2539-2547. doi:10.1161/ATVBAHA.120.314872
26. Elshazli R, Toraih E, Elgaml A, El-Mowafy M, El-Mesery M, Amin M et al. Diagnostic and prognostic value of hematological and immunological markers in COVID-19 infection: A meta-analysis of 6320 patients. *PLoS One*. 2020;15(8): e0238160. doi:10.1371/journal.pone.0238160
27. Simadibrata DM, Lubis AM. D-dimer levels on admission and all-cause mortality risk in COVID-19 patients: a meta-analysis. *Epidemiol Infect*. 2020; 148:e202. doi:10.1017/S0950268820002022
28. Baroiu L, Lese A, Stefanopol I, Iancu A, Dumitru C, Ciubara A et al. The Role of D-Dimers in the Initial Evaluation of COVID-19. *Ther Clin Risk Manag*. 2022;18:323-335. doi:10.2147/TCRM.S357214
29. Düz M, Balcı A, Menekşe E. D-dimer levels and COVID-19 severity: Systematic Review and Meta-Analysis. *Tuberk Toraks*. 2020;68(4):353-360. doi:10.5578/tt.70351
30. Del Sole F, Farcomeni A, Loffredo L, Carnevale R, Menichelli D, Vicario T et al. Features of severe COVID-19: A systematic review and meta-analysis. *Eur J Clin Invest*. 2020;50(10):e13378. doi:10.1111/eci.13378
31. Terpos E, Ntanasis-Stathopoulos I, Elalamy I, Kastritis E, Sergentanis T, Politou M et al. Hematological findings and complications of COVID-19. *Am J Hematol*. 2020;95(7):834-847. doi:10.1002/ajh.25829
32. Rostami M, Mansouritorghabeh H. D-dimer level in COVID-19 infection: a systematic review. *Expert Rev Hematol.* 2020;13(11):1265-1275. doi:10.1080/17474086.2020.1831383
33. González JJ. D-dimer pitfalls and performance in COVID-19. *Med Crit*. 2020;34(4):214-215. doi:10.35366/95875
34. Yao Y, Cao J, Wang Q, Shi Q, Liu K, Luo Z et al. D-dimer as a biomarker for disease severity and mortality in COVID-19 patients: a case control study. *J Intensive Care*. 2020;8:49. doi:10.1186/s40560-020-00466-z
35. Huang I, Pranata R, Lim M, Oehadian A, Alisjahbana B. C-reactive protein, procalcitonin, D-dimer, and ferritin in severe coronavirus disease-2019: a meta-analysis. *Ther Adv Respir Dis*. 2020; 14:1753466620937175. doi:10.1177/1753466620937175
36. Chi G, Lee J, Jamil A, Gunnam V, Najafi H, Memar Montazerin S et al. Venous Thromboembolism among Hospitalized Patients with COVID-19 Un-

dergoing Thromboprophylaxis: A Systematic Review and Meta-Analysis. *J Clin Med*. 2020;9(8): 2489. doi:10.3390/jcm9082489
37. Gong J, Dong H, Xia Q, Huang Z, Wang D, Zhao Y et al. Correlation analysis between disease severity and inflammation-related parameters in patients with COVID-19: a retrospective study. *BMC Infect Dis*. 2020;20(1):963. doi:10.1186/s12879-020-05681-5
38. Poudel A, Poudel Y, Adhikari A, Aryal B, Dangol D, Bajracharya T et al. D-dimer as a biomarker for assessment of COVID-19 prognosis: D-dimer levels on admission and its role in predicting disease outcome in hospitalized patients with COVID-19. *PLoS One*. 2021;16(8):e0256744. doi:10.1371/journal.pone.0256744
39. Soni M, Gopalakrishnan R, Vaishya R, Prabu P. D-dimer level is a useful predictor for mortality in patients with COVID-19: Analysis of 483 cases. *Diabetes Metab Syndr*. 2020;14(6):2245-2249. doi:10.1016/j.dsx.2020.11.007
40. Gonçalves FA, Besen BA, Lima CA, Corá AP, Pereira AJ, Perazzio SF et al. Use and misuse of biomarkers and the role of D-dimer and C-reactive protein in the management of COVID-19: A post-hoc analysis of a prospective cohort study. *Clinics (Sao Paulo).* 2021;76:e3547. doi:10.6061/clinics/2021/e3547
41. Lippi G, Favaloro E. D-dimer is Associated with Severity of Coronavirus Disease 2019: A Pooled Analysis. *Thromb Haemost*. 2020;120(5):876-878. doi:10.1055/s-0040-1709650
42. Sukrisman L, Sinto R. Coagulation profile and correlation between D-dimer, inflammatory markers, and COVID-19 severity in an Indonesian national referral hospital. *J Int Med Res*. 2021; 49(11):3000605211059939. doi:10.1177/03000605211059939
43. Bansal A, Singh A, Jain V, Aggarwal M, Gupta S, Padappayil R et al. The association of D-dimers with mortality, intensive care unit admission or acute respiratory distress syndrome in patients hospitalized with coronavirus disease 2019 (COVID-19): A systematic review and meta-analysis. *Heart Lung*. 2021;50(1):9-12. doi:10.1016/j.hrtlng.2020.08.024
44. Naymagon L, Zubizarreta N, Feld J, van Gerwen M, Alsen M, Thibaud S et al. Admission D-dimer levels, D-dimer trends, and outcomes in COVID-19. *Thromb Res*. 2020;196:99-105. doi:10.1016/j.thromres.2020.08.032
45. Kermali M, Khalsa R, Pillai K, Ismail Z, Harky A. The role of biomarkers in diagnosis of COVID-19 - A systematic review. *Life Sci*. 2020;254:117788. doi:10.1016/j.lfs.2020.117788
46. Nugroho J, Wardhana A, Maghfirah I, Mulia E, Rachmi D, A'yun M et al. Relationship of D-dimer with severity and mortality in SARS-CoV-2 patients: A meta-analysis. *Int J Lab Hematol*. 2021; 43(1):110-115. doi:10.1111/ijlh.13336
47. Shah S, Shah K, Patel S, Patel F, Osman M, Velagapudi P et al. Elevated D-Dimer Levels Are Associated With Increased Risk of Mortality in Coronavirus Disease 2019: A Systematic Review and Meta-Analysis. *Cardiol Rev*. 2020;28(6):295-302. doi:10.1097/CRD.0000000000000330
48. Chaudhary R, Garg J, Houghton D, Murad M, Kondur A, Chaudhary R et al. Thromboinflammatory Biomarkers in COVID-19: Systematic Review and Meta-analysis of 17,052 Patients. *Mayo Clin Proc Innov Qual Outcomes.* 2021;5(2):388-402. doi:10.1016/j.mayocpiqo.2021.01.009
49. Sakka M, Connors J, Hékimian G, Martin-Toutain I, Crichi B, Colmegna I et al. Association between D-Dimer levels and mortality in patients with coronavirus disease 2019 (COVID-19): a systematic review and pooled analysis. *J Med Vasc*. 2020; 45(5):268-274. doi:10.1016/j.jdmv.2020.05.003
50. PCN-371. Infecția cu coronavirus de tip nou (COVID-19), ediția VIII. 2023. 97 p.
51. Mouhat B, Besutti M, Bouiller K, Grillet F, Monnin C, Ecarnot F et al. Elevated D-dimers and lack of anticoagulation predict PE in severe COVID-19 patients. *Eur Respir J*. 2020;56(4):2001811. doi:10.1183/13993003.01811-2020

Date of receipt of the manuscript: 23/11/2022
Date of acceptance for publication: 11/07/2023

OH&RM ONE HEALTH & RISK MANAGEMENT

VOL. 4, ISSUE 4
2023

MEDICAL SCIENCES

OPEN ACCESS

RISC MANAGEMENT: THE MEDICAL SUPPORT SYSTEM IN CONTEMPORARY ARMED CONFLICTS

Sergiu CIRLAN[1], Andrei MARFIN[2], Vasile DUMITRAS[3]

[1]Military Academy of the Armed Forces "Alexandru cel Bun", Chisinau, Republic of Moldova
[2]Outpatient Clinic of Ministry of Defence, Chisinau, Republic of Moldova
[3]*Nicolae Testemitanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova

Corresponding author: Sergiu Cirlan, e-mail: scirlan@yahoo.com

DOI: 10.38045/ohrm.2023.4.06 CZU: 355.415.6

Keywords: *armed conflict, medical support, deployment, medical service, Roles.*

Introduction. *Contemporary armed conflicts are characterized by a multifaceted blend of combat strategies, encompassing conventional and unconventional weaponry, regular and irregular forces, terrorist actions and acts of organized crime, indiscriminate violence and outside international law.*

Material and methods. *A retrospective bibliographic study was conducted on the evolution of combat strategies employed by the U.S. Armed Forces, NATO member countries, and international UN Peacekeeping Operations over the past 25 years.*

Results. *The way of organizing and providing medical assistance in local military conflict differs from the principles governing medical support in major armed conflicts. The staging of the treatment system and the evacuation of the wounded follows a crucial time-based framework known as the '10-1-2(+2)' rule: "10"– immediate measures to stop bleeding within 10 minutes of the injury; "1" – evacuation to the medical treatment facility capable of performing resuscitation and stabilizing vital functions.; "2" – timely transfer to a medical formation equipped for Damage Control Surgery and Resuscitation within 2 hours of the injury; "+2" – performing the surgical intervention, stabilization of vital functions, and diagnostic preparations for strategic evacuation within 4 hours of the injury.*

Conclusions. *Resulting from the specific particularities of the local military conflict, the planning of medical support as well as the complex of treatment and evacuation measures, force health protection, medical logistics, require adaptation to the real tactical and medical situation created.*

Cuvinte-cheie: *conflict militar, sprijin medical, eşalonarea, serviciu medical, Role.*

GESTIONAREA RISCURILOR: SISTEMUL DE SPRIJIN MEDICAL ÎN CONFLICTUL ARMAT CONTEMPORAN

Introducere.*-Conflictul militar contemporan poate fi caracterizat ca o combinaţie a diverselor moduri de de luptă, utilizând armele convenţionale şi neconvenţionale, forţe regulate şi neregulate, acţiuni teroriste şi alte acte de criminalitate organizată, violenţă nediscriminatorie şi alte acţiuni care contravin dreptului internaţional..*

Material şi metode. *A fost realizat un studiu bibliografic retrospectiv din ultimii 25 de ani a evoluţiei modului de luptă ale Forţelor Armate ale SUA, ţărilor membre NATO, precum şi operaţiunile internaţionale de menţinere a păcii ONU.*

Rezultate. *Modul de organizare şi acordare a asistenţei medicale în conflictul militar local diferă de la principiile sprijinului medical în conflictul militar major. Eşalonarea sistemului de tratament şi evacuare a răniţilor se bazează pe imperativul intervalului recunoscut ca „10-1-2(+2)": „10" – controlul căilor respiratorii şi hemostaza provizorie – până la 10 minute de la momentul rănirii; „1" – evacuarea la formaţiunea medicală cu capabilităţile de efectuare a resuscitării/stabilizării funcţiilor vitale; „2" – evacuarea la formaţiunea medicală care asigură asistenţa chirurgicală de urgenţă – până la 2 ore de la momentul rănirii; „+2" – efectuarea intervenţiei chirurgicale, stabilizarea funcţiilor vitale, diagnosticarea rectificată vizând pregătirea răniţilor pentru evacuare strategică – până la 4 ore de la momentul rănirii.*

Concluzii. *Reieşind din particularităţile specifice ale conflictului militar local, planificarea sprijinului medical cât şi complexul de măsuri de tratament şi de evacuare sanitaro-igienice şi antiepidemice, logistica medicală necesită adaptare la situaţia tactică şi medicală reală creată.*

INTRODUCTION

The changing landscape of armed conflicts in the past three decades has given rise to novel trends and diverse scenarios in the preparation and execution of combat operations. Consequently, military strategies have undergone significant modifications (1). The advent of cutting-edge technologies has ushered in a revolutionary shift in the methods and approaches employed in combat actions. Modern armed conflicts are distinguished by the fusion of various modes of engagement: encompassing traditional and unconventional weaponry, both regular and irregular forces, instances of terrorism, organized criminal activities, indiscriminate violence, and actions that operate beyond the international law (2). In the majority of contemporary armed conflicts, operational military forces typically consist of task forces ranging from 1-2 battalions up to a brigade, responsible for autonomous and self-sustained combat operations. Insights gleaned from recent operational experiences underscore a discernible contrast in the structure and delivery of medical assistance between regional military conflicts and large-scale armed confrontations.

The aim of the study is to highlight the particularities related to the changing nature of armed conflicts and their impact on military strategies, technological advances, and medical support systems.

MATERIAL AND METHODS

A comprehensive retrospective bibliographic study was conducted to trace the evolution of combat action methodologies within the United States of America, NATO member nations, and international UN Peacekeeping Operations over the past 25 years.

RESULTS

Contemporary armed conflicts are distinguished by the execution of combat actions utilizing resources and capacities aligned with peacetime organizational structures, often involving partial mobilization. These conflicts typically occur within confined operational zones, with the timeframe for conducting combat actions spanning anywhere from as short as 6 days (as seen in the 1967 Arab-Israeli conflict) to as extended as 8 years (as observed in the 1980-1988 Iran-Iraq conflict). The intensity of combat actions during such conflicts displays cyclic fluctuations, marked by the gradual accumulation of forces and resources over a span of up to 6 months, leading to direct combat engagements lasting around 7 days. Importantly, the civilian populace residing in the involved belligerent parties is exposed to substantial risks stemming from detrimental factors, resulting in the unfortunate occurrence of collateral losses (2).

The core concept of the modern staged treatment and evacuation system lies in the sequential implementation of treatment procedures, following a specific order of medical stages. These stages are closely linked with the transfer of wounded or sick individuals to specialized medical units, aligning with their medical requirements and designated destinations. Additionally, this approach factors in the dynamic interplay between the prevailing combat circumstances and the medical settings (3, 4, 5).

As per the stipulations of the NATO Directive of 2010, the previously emphasized critical time frame for delivering medical assistance known as the “Golden Hour” has been replaced by the '10-1-2 (+2)' interval, comprising:

- "10" – stop bleeding - up to 10 minutes from the time of injury;
- "1" – evacuation to the medical treatment facility capable of performing resuscitation/stabilization of vital functions;
- "2" – evacuation to the medical formation that provides Damage Control Surgery and Resuscitation – up to 2 hours from the time of injury;
- "+2" – surgical intervention, stabilization of vital functions, diagnosis aimed at preparing the wounded for strategic evacuation (STRATEVAC) – up to 4 hours from the time of injury.

Taking into account the ”optimal range” and the capacities of medical units within the Armed Forces of NATO member countries and the United States of America, the medical service assets and resources are organized into four distinct levels: Role 1, 2, 3, and 4. These medical units are categorized into Roles based on their minimum clinical and paraclinical capabilities (tab. 1). Additionally, it's noteworthy that the operational readiness and mobility of these medical units align with the military structures they support.

Table 1. The capabilities of Role 1 (R1) level medical treatment facility.

The capabilities	Ensures the primary medical assistance of the workforce, including first aid (medical aid), triage, resuscitation and stabilization activities.
	Ensures the retrieval of the wounded individuals and their readiness for evacuation.
	Provides elements of preventive medicine.
	Provides a medical protection component against weapons of mass destruction, probably used by the adversary.
	May include minimal capacities for providing short-term patient care, primary dental services, a laboratory for conducting essential analyses, and personnel trained in combat stress management.
	Can allocate forces and means for the medical support of the forces, whether from advanced positions or isolated locations.
	Can allocate forces and means to establish rapid response medical teams.

The Role 1 medical treatment facility encompasses the Battalion Aid Station (BAS), which serves as the fundamental component of the battalion's medical platoon.

The organizational structure of the medical platoon comprises the following components: management, treatment unit, ambulance unit, and health instructors' unit. The Commander, treatment and ambulance units together form the Battalion Aid Station within the battalion.

The treatment unit serves as a fundamental component in delivering medical aid at the battalion level. The unit's personnel are organized into two groups:

- group "A" consists of a doctor and up to three nurses.
- group "B" comprises a paramedic and up to three health instructors.

The ambulance unit comprises two ambulance teams: a tracked ambulance team with three members and a wheeled ambulance team with two members. This section is responsible to transport the wounded from the injury site or Casualty Collection Point (CCP) to the Battalion Aid Station (BAS).

The combat medic unit consists of combat medics, each assigned to a maneuver company.

The Role 2 (R2) medical treatment facility possesses the capability to receive and prioritize wounded individuals, as well as provide advanced resuscitation and treatment for traumatic shock (tab. 2).

Table 2. The types of Role 2 and their capabilities (6).

The types	**The capabilities**
Role 2 Forward (R2F) – the advanced medical formation, with high mobility *(The resources of the given formation are limited)*	advanced emergency surgical assistance
	postoperative care
	preparing the wounded for further evacuation
Role 2 Basic (R2B) – the mobile medical treatment facility (*Limitations in the hospitalization of the wounded and medical supply constraints)*	reception
	triage
	resuscitation and emergency surgery
	postoperative care
Role 2 Enhanced (R2E) – strengthened medical treatment facility	diagnostic
	specialized medical assistance
	prepare the wounded for strategic evacuation (STRATEVAC)

The unit can be deployed independently to enhance the delivery of medical assistance within the operational theatre.

The medical support concept of the United States Armed Forces dictates that each branch or type of service possesses its own Role 2 medical formations.

Within the Land Forces, these formations consist of the medical company and the Advanced Surgical Team.

There are two types of medical companies, each carrying out the same missions, but differing in their operational areas:

1. The Brigade Medical Support Company, also referred to as the "Charlie Company", operating within the Brigade Support Battalion, offers medical assistance at Role 1 and 2 levels to personnel within the tactical group at the brigade level, including the reinforced units. Depending on the situation, the company can establish up to four medical teams, each comprising a surgeon, three paramedics, and three nurses, capable of autonomous operation.
2. The Area Support Medical Company is an integral part of a medical battalion within the medical brigade.

The company creates medical teams intended for deployment within the division support brigade. These teams perform operations in the rear area of the division or at a corps logistics group. The primary mission is to provide medical aid to operational units that lack internal medical formations within their organizational structure (6).

In light of the insights gained from the "Desert Storm" operation in 1991, a decision was made to eliminate the Mobile Army Surgical Hospital (MASH) from the division-level treatment and evacuation system.

The hospital's responsibilities were handed over to the Forward Surgical Team (FST), whose mission is to provide surgical assistance to injured individuals unable to be transported to the next stage due to conditions such as severe bleeding or respiratory distress.

This team comprises 20 members, including three general surgeons, a traumatologist, two anesthesiology nurses, and three general nurses.

The FST has the capacity to conduct 20 surgical procedures daily, with subsequent intensive care lasting up to 6 hours per patient. Overall, intensive therapy can be provided to a maximum of 8 injured individuals simultaneously, for a duration of up to 72 hours (7, 8).

Surgical aid for the wounded adheres to the "Damage control" principle, focusing on surgical interventions aimed at preserving the lives of the injured individuals.

The term "Damage control," derived from the American maritime terminology, signifies the use of all available resources in the effort to rescue a sinking vessel.

The approach of initially planned surgical treatment was employed to reduce mortality rates among individuals with abdominal injuries, particularly those complicated by significant hemorrhaging. This approach aims to avert organ evisceration and postpone the repair of injured organs until the patient's vital functions have been stabilized.

The Role 3 (R3) level medical treatment facility encompasses the following capabilities:

1. This medical formation is designed to deliver secondary medical care, possessing the capacity to admit and retain severely injured or critically ill patients within a hospital setting. It is fully equipped to conduct comprehensive diagnoses and deliver specialized surgical treatments and recovery services for injuries sustained in combat. Depending on the prevailing operational protocols, patients may also be transferred to a Role 4 Medical Treatment Facility (MTF). The R3 facility is staffed with experts in essential surgical disciplines, anesthesia-intensive therapy, internal medicine, infectious diseases, ophthalmology, neurology, and others, as necessitated by the mission. The equipment is tailored to suit the specific requirements of the overall task.
2. The facility provides services for blood transfusion and blood substitutes.
3. Depending on specific needs, the facility may include specialized departments with experts in neurosurgery, oral-maxillo-facial surgery, and advanced medical imaging techniques such as computed tomography, ultrasound, arthroscopy, and more (9).

The medical units within the Role 3 echelon constitute the apex of medical aid accessible within the operational theatre, offering the most extensive hospitalization capabilities.

These medical formations are designed to operate at the army corps level and are represented by the Combat Support Hospital. Their core mission is to deliver comprehensive medical support, encompassing both outpatient and inpatient care, to all patient categories within the Operation Theatre.

The hospital structure comprises:

1. Management, along with a management detachment.
2. Hospital Company with 84 beds (Alpha Co).
3. Hospital Company with 164 beds (Bravo Co).

The"Alpha" company (44 beds) comprises the following capabilities: 2 operating rooms (with a maximum capacity of 36 hours for surgical interventions per day), 2 intensive care units (each with 12 beds), and a hospital unit (20 beds). In addition to the Hospital Reinforcement Element's capacity (20 intensive care beds), it also constitutes a Field Hospital (84 beds).

The capabilities of the 'Bravo' hospital company (164 beds) encompass: 4 operating rooms (with a maximum capacity of 60 hours for surgical interventions per day), 2 intensive care units (each having 12 beds), and 7 inpatient units (each equipped with 20 beds) (10).

A hospital ship (Hospital Ship) with a capacity of 1000 beds (including 100 intensive care beds and 12 operating rooms) is designated for providing medical assistance within the Naval Forces at the Role III level (11 - 14).

The Role 4 (R4) medical treatment facility encompasses a wide range of specialties, investigations, and medical procedures. This medical unit operates within the national territory, at its designated permanent deployment location.

CONCLUSIONS

1. While a significant portion of today's global operations involve peacekeeping missions, it's important to acknowledge that there are various other types of military conflicts in their active phases as well.
2. Due to the unique characteristics of local military conflicts, the planning of medical support , along with the intricate set of treatment and evacuation measures, force health protection, and medical logistics, necessitates adaptation to the actual tactical and medical circumstances .
3. This imperative calls for the integration of contemporary approaches methods in medical support, spanning from the battlefield to the recovery of the wounded and sick.

CONFLICT OF INTERESTS

Authors declare that they do not have conflicts of interest.

ETHICAL APPROVAL

The article was not approved by the Ethics Committee because it does not contain ethical risks.

REFERENCES

1. Global Trends in Armed Conflict Events, 1946-2016. *Assessing the Qualities of Systemic Peace*. Available at: http://www.systemicpeace.org/conflicttrends.html#fig2/ [Accessed 25.05.2023].
2. Stan A, Sarcinschi A, Siteanu E. *Conflictele atipice ale secolului al XXI-lea* [The atypical conflicts of the 21st century]. Editura Universităţii Naţionale de Apărare „Carol I". Bucureşti, 2015. Available at: https://cssas.unap.ro/ro/pdf_studii/conflictele_atipice_ale_secolului_XXI.pdf [Accessed 05.04.2023].
3. Md-2, Doctrina sprijinului medical în operaţii întrunite. Bucureşti [Doctrine of medical support in joint operations. Bucharest], 2016.
4. AJP 4.10 Allied Joint Doctrine for Medical Support. Brussels, 2019. Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/922182/doctrine_nato_med_spt_ajp_4_10.pdf [Accessed 09.04.2023].
5. ACO Directive (AD) 83-1 (Edition 2) Medical Support to Operations. Brussels.2010.
6. FM 8-10-4. Medical Platoon Leaders handbook, tactics, techniques, and procedures. Washington, DC, 2001. Available at: https://documentafterlives.newmedialab.cuny.edu/content/army-field-manual-no-4-024-fm-4-024-fm-8-10-4-medical-platoon-leaders-handbook-tactics [Accessed 10.03.2023].
7. FM 8-10-1. The medical company, tactics, techniques, and procedures. Washington, DC, 2002.

8. FM 4-02.25. Employment of Forward Surgical Teams tactics, techniques, and procedures. Washington, DC, 2003. Available at: https://archive.org/details/ost-military-doctrine-fm4_02x25 [Accessed 10.03.2023].
9. Medical Support manual for Unated Nationas Field Missins, 3 edition. UN. New York. 2015. Available at: https://tactical-medicine.com/blogs/news/medical-support-manual-for-united-nations-field-missions-2015 [Accessed 10.03.2023].
10. FM 4-02.10. Theater Hospitalisation. Washington, DC,2020. Available at: https://armypubs.army.mil/epubs/DR_pubs/DR_a/ARN30125-ATP_4-02.10-000-WEB-1.pdf [Accessed 10.03.2023].
11. Richard T, Peacock T, Cubano M, Elster E, Gurney J, Charlton M, White P, Schmidt C, Gutierrez L. *Emergency War Surgery*. AMSUS, 2018. Available at: https://www.amsus.org/wp-content/uploads/2018/12/AMSUS-EWS-Final-29-Nov-18.pdf [Accessed 19.05.2023].
12. MC 326/2 NATO Medical Support Principles and Policies. Brussels. 2009. Available at: https://www.sto.nato.int/publications/STO%20Meeting%20Proceedings/RTO-MP-HFM-109/MP-HFM-109-$KN2.pdf [Accessed 19.05.2023].
13. FM 4-02.2 (8-10-26 and 8-10-6). Medical Evacuation. Washington, DC, 2007. Available at: https://irp.fas.org/doddir/army/fm4-02-2.pdf [Accessed 19.05.2023].
14. FM 4-02.24. Area Support Medical Battalion - Tactics, Techniques, and Procedures. Washington, DC, 2000.

Date of receipt of the manuscript: 28/06/2023
Date of acceptance for publication: 17/09/2023

OH&RM ONE HEALTH & RISK MANAGEMENT

VOL. 4, ISSUE 4
2023

RESEARCH ARTICLE – ARTICLES DE RECHERCHE

MEDICAL SCIENCES

OPEN ACCESS

A REDUCED FIXED DOSE OF TOCILIZUMAB 200 MG COMPARED TO 400 MG IN PATIENTS WITH SEVERE COVID-19 DISEASE

Stela COJOCARU[1], Irina RUSSU[1], Galina BUTA[1], Sebastian BERSAN[1], Valentina POTING-RASCOV[1], Natalia CULIUC[2], Lilia BABA[2]

[1]*Nicolae Testemitanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova
[2]*Toma Ciorba* Clinical Hospital of Infectious Diseases, Chisinau, Republic of Moldova

Corresponding author: Irina Russu, e-mail: irina.rusu@usmf.md

DOI: 10.38045/ohrm.2023.4.02 CZU: [616.98:578.834.1]-085.2

Keywords: *COVID-19, Cytokine Storm, Tocilizumab, severe forms, oxygen therapy.*

Introduction. *Excessive, deregulated pro-inflammatory cytokine secretion has a detrimental impact on the evolution of COVID-19, aggregating the tissue impairment, organ failure, and an increased risk of death. Several studies have demonstrated the beneficial effect of Tocilizumab (TCZ) in reducing hyperimmune response in severe forms of COVID-19.* ***Material and methods.*** *This is an experimental controlled clinical trial, consisting of 66 patients hospitalized with severe COVID-19.* ***Results.*** *On overage, the decision to administer TCZ was made on average on the 11.34±0.31 day of the disease, when the beginning of Cytokine Storm was suspected in the patients already on dexamethasone treatment. The clinical and paraclinical parameters, including fever, asthenia and dyspnea duration, SpO2 level, oxygen therapy need, improvement of the radiological picture, and duration of hospitalization were more favorable in patients treated with TCZ 400 mg compared to those treated with TCZ 200 mg ($p<0.0001$). The relative risk of rapid worsening after TCZ (RR=0.88), the relative risk of decreasing blood pressure (RR=0.29) and the relative risk of transfer to intensive care units for invasive or non-invasive ventilation (RR=0.8) was lower in patients treated with TCZ 200 mg compared to the 400 mg TCZ lot.* ***Conclusions.*** *The dose of TCZ had a significant impact on the duration of clinical manifestations, the duration of oxygen therapy and the duration of patient hospitalization, with better results for TCZ 400 mg compared to TCZ 200 mg. Although the risk of worsening after TCZ and the risk of transfer to intensive care were lower in patients treated with TCZ 200 mg. So, the 200 mg fixed dose of TCZ can be a life-saving option for severely ill patients with COVID-19 in the context of IL-6 inhibitor supply shortages.*

Cuvinte-cheie: *COVID-19, furtună de citokine, Tocilizumab, forme severe, terapie cu oxigen.*

DOZĂ REDUSĂ DE TOCILIZUMAB 200 MG, COMPARATIV CU 400 MG, LA PACIENȚII CU FORME SEVERE DE INFECȚIE COVID-19

Introducere. *Eliberarea excesivă a citokinelor proinflamatorii are un impact negativ asupra evoluției infecției COVID-19, sporind afectarea tisulară, insuficiența organică și riscul de deces. Mai multe studii au demonstrat efectul benefic al preparatului Tocilizumab (TCZ) în reducerea răspunsului hiperimun în formele severe de infecție COVID-19.* ***Material și metode.*** *Este un studiu experimental, clinic controlat, care include 66 pacienți, internați cu forme severe de infecție COVID-19.* ***Rezultate.*** *Decizia privind administrarea de TCZ a fost luată în medie în ziua a 11,34±0,31 de boală, când a fost suspectat debutul furtunii de citokine la pacienții aflați deja în tratament cu dexametazonă. Parametri clinici și paraclinici, precum durata febrei, asteniei și a dispneei, nivelul SpO_2, necesitatea în terapie cu oxigen, ameliorarea tabloului imagistic radiologic, și durata spitalizării au fost mai favorabili la pacienții tratați cu TCZ 400 mg față de cei tratați cu TCZ 200 mg ($p<0.0001$). Riscul relativ de agravare rapidă după administrarea TCZ (RR=0,88), riscul relativ de diminuare a tensiunii arteriale (RR=0,29) și riscul relativ de transfer în secțiile de terapie intensivă pentru ventilație invazivă sau non-invazivă (RR=0,8) a fost mai scăzut la pacienții tratați cu TCZ 200 mg, comparativ cu lotul TCZ 400 mg.* ***Concluzii.*** *Doza de TCZ a influențat durata manifestărilor clinice, durata terapiei cu oxigen și durata spitalizării pacienților, cu rezultate mai bune pentru TCZ 400 mg față de TCZ 200 mg, deși riscul de agravare după TCZ și riscul de transfer în terapie intensivă au fost mai joase la pacienții tratați cu TCZ 200 mg. Deci, doza de TCZ de 200 mg poate fi o opțiune de salvare a vieții pacienților gravi cu infecție COVID-19, în contextul deficitului de aprovizionare cu inhibitori de IL-6.*

INTRODUCTION

The COVID-19 pandemic caught the medical community by surprise and totally unprepared. After two years of brainstorming, COVID-19 still has many unclarified pathogenetic and therapeutic issues. Cytokines are essential for tissue homeostasis and, as mediators, unleash an effective immune response during infections (1, 2). However, the deregulated excessive pro-inflammatory cytokine secretion has a detrimental impact on the evolution of COVID-19, aggregating the hemodynamic alterations, tissue impairment, organ failure, and increasing the risk for death (3, 4, 5).

It is considered that COVID-19 evolves in several stages, each with its specific pathogenetic and clinical characteristics: early stage – marked by high multiplication of SARS CoV-2; stage IIA – distinct by the appearance of pulmonary pathology; stage IIB – defined by pulmonary involvement with hypoxia and the beginning of systemic inflammation; stage III – expressed by evolution to extrapulmonary systemic hyper inflammation syndrome (6). Interestingly, there is no concluding association between the clinical aggravation of the patients in the second week of the disease and viral load, supporting the primordial role of the immune response in COVID-19 severity (7). Thus, for clinicians is extremely important to understand all pathophysiologic stages in the disease's evolution and correctly appreciate the appropriate moment for antivirals, corticosteroids, and cytokine inhibitors administration.

It is commonly considered that IL-6 plays the fundamental role among pro-inflammatory cytokines in the progression of SARS CoV-2 infection (8, 9, 10). IL-6 signalling in the vascular endothelium has a key role in the imbalance toward the prothrombotic state (11, 12, 13). Pulmonary inflammation could trigger local vascular dysfunction and fibrinolysis, thus contributing to fibrin deposition and dysfunction of alveolar-capillary blood gas exchange (14, 15). In this setting, several studies have demonstrated the beneficial effect of IL-6 inhibitors in reducing hyperimmune response in severe forms of COVID-19 (16 - 20). The therapeutic effect of IL-6 inhibitors in COVID-19 patients with exaggerated systemic inflammatory response was investigated in randomized, controlled platform trials, RECOVERY, and REMAP-CAP, that revealed its beneficial ef fect on the survival rate (21, 22). These studies prompted the introduction of Tocilizumab (TCZ), a monoclonal antibody that inhibits the Interleukin-6 (IL-6) receptor in the treatment guidelines for severe COVID-19 (23 - 26). Reminding the pathogenetic stages of disease evolution in SARS CoV-2 infection is essential to realize the significance of the time frame of TCZ administration during COVID-19: not too early, but not too late. Thus, IL-6 inhibitors may not give additional benefits while the patient is already in a critical state and require invasive mechanical ventilation (27, 28). In most studies, TCZ is administered to patients already severely hypoxemic, requiring mechanical ventilation, even in a critical state or within the first hours of life support measures such as invasive or non-invasive ventilation. This may partially be the cause of the lack of plausible results of TCZ treatment in some trials of COVID-19 (29, 30, 31).

Taking into consideration the pathogenesis of excessive systemic inflammatory immune response in severe cases of SARS CoV-2 infection, Tocilizumab was the second drug highly suggested by the WHO for COVID-19 severe or critical cases treatment after dexamethasone recommendation in September 2020. Administration of IL-6 inhibitors for COVID-19 has been prequalified from rheumatoid arthritis (RA) and systemic juvenile idiopathic arthritis (SJIA) treatment schedules. The recommended dose of TCZ accepted by most guidelines for COVID-19 is 8 mg/kg up to a maximum of 800 mg, as an intravenous bolus infusion, and a second dose administration can occur 12 to 48 hours later. Other studies proved that a dose of TCZ 400 mg demonstrates an equivalent reduction of inflammation and comparable mortality to a dose of 8 mg/kg (32). The COVIDOSE study concluded that TCZ 4–8 mg/kg dose might be too much for patients with COVID-19 hyper inflammation syndrome. The dose-finding studies, especially of minimal acceptance, and the administration time frame that can provide a valuable anti-inflammatory effect in COVID-19 hypercytokine response have not yet been fully completed.

The study aimed to compare physiological responses and clinical outcomes of IV TCZ 200 mg vs. 400 mg fixed dose bolus in patients with severe COVID-19 that do not require invasive ventilation at the moment of administration. The study was also reasoned by the shortages of IL-6 inhibi-

tors supply in the Republic of Moldova during the pandemic period.

MATERIAL AND METHODS

A controlled experimental clinical trial was conducted. This study was carried out with the approval of the Ethics Committee no. 02/02.10.2020 of the *Nicolae Testemitanu* State University of Medicine and Pharmacy, Republic of Moldova. After obtaining the patients' agreement to participate in the research, two groups of patients with COVID-19 infection, severe form, hospitalized between December 2020 and June 2021 in the Toma Ciorba Clinical Hospital for Infectious Diseases, Republic of Moldova, were made. A total of 66 patients were included in the study. Group ratio 1:1 (L1=33, L2=33). Patients in the research group (L1) were treated with a single intravenous dose of Tocilizumab at 200 mg. Those in the control group (L2) received a single intravenous dose of Tocilizumab at 400 mg. The TCZ dose decision was made randomly.

The diagnosis of COVID-19 was confirmed by detecting SARS-CoV-2 RNA by PCR tests. Patients were not COVID-19 vaccinated, and no one received monoclonal antibodies targeting the spike glycoprotein or antiviral treatment against SARS CoV-2 during the study period. Criteria for inclusion in the study were: people over the age of 18 with severe forms of COVID-19 who developed signs of cytokinic storm. The data from the patients' medical records were processed according to a unified, pre-established form that included epidemiological, socio-demographic, clinical, paraclinical, laboratory data, and information on therapeutic management. Data were collected from the day of hospitalization till the date of discharge or transfer to ICU.

A decision regarding TCZ administration was made when the beginning of Cytokine Storm (hypercytokinemia) was suspected in the patients already on dexamethasone treatment.

Criteria for TCZ administration were the combination of fever >38°C, prolonged or reappearance in the second wave, exacerbation of asthenia, intensification of dyspnea, advancing hypoxemia SpO_2 ≤94% at rest on room air, bilateral lung involvement with progressive negative evolution of imaging over the last 24-48 hours, requirement of oxygen supplementation 10 L/min within 24-48 hours of commencement of respiratory support on nasal oxygen, absence of general improvement following treatment with corticosteroids (dexamethasone).

Exclusion criteria for the study group were: age below 18 years old, pregnancy, pre-existing treatment resulting in ongoing immunosuppression, co-existing diseases that might be worsened by IL-6 inhibitors, such as systemic fungal or bacterial co-infection, patients in shock, a necessity in non-invasive/invasive ventilation before TCZ administration, patients with poor prognosis indicating an unlikely survival of over 48 h.

Clinical and paraclinical parameters of patients at admission and during treatment, the presence of comorbidities, the persistence of fever after treatment with TCZ and its duration, the rate of patients with low oxygenation (SpO_2 <94%) and the terms of improvement of O_2 saturation, the proportion of patients transferred to intensive care department for invasive or non-invasive ventilation, as well as the duration of hospitalization were evaluated. The accumulated data were entered into Excel. The statistical evaluation of the data obtained was performed using the MedCalc program. To estimate the efficacy of treatment in the compared groups, tests were evaluated: Arithmetic mean ± mean error (M±m), p-significance coefficient ($p<0.05$), Relative Risk (RR), 95% Confidence Interval (95%CI) and Number Needed to Treat (NNT).

RESULTS

The average age of the patients included in the study was 58.22±1.38 years, L_1 group - 58.6±2.3 y/o, L_2 group - 57.8±1,6 [95%CI (0.17-1.77), p=0.11]. The male/female ratio in the total group was 0.83, L_1 group – 0.5 and L_2 group – 1.35. Feminine gender predominates in the L_1 group – 22 (66.7%) patients, L_2 group – 14 (42.4%) patients [95%CI (0.99-7.38), p=0.05], while masculine gender predominate in the L_2 group – 19 (57.6%) patients, L_1 group – 11 (33.3%) patients [95%CI (0.14-1.0), p=0.05]. Contact with the patients with COVID-19 was confirmed in 28 (42.4%) cases.

Patients were hospitalized during the different periods of disease evolution. The average day of hospitalization from the onset of the disease was 7.86±0.38 (2-14) days. The main clinical manifestations of the patients in the total group while en tering the hospital were fever, fatigue, cough, dyspnea and headache (fig. 1).

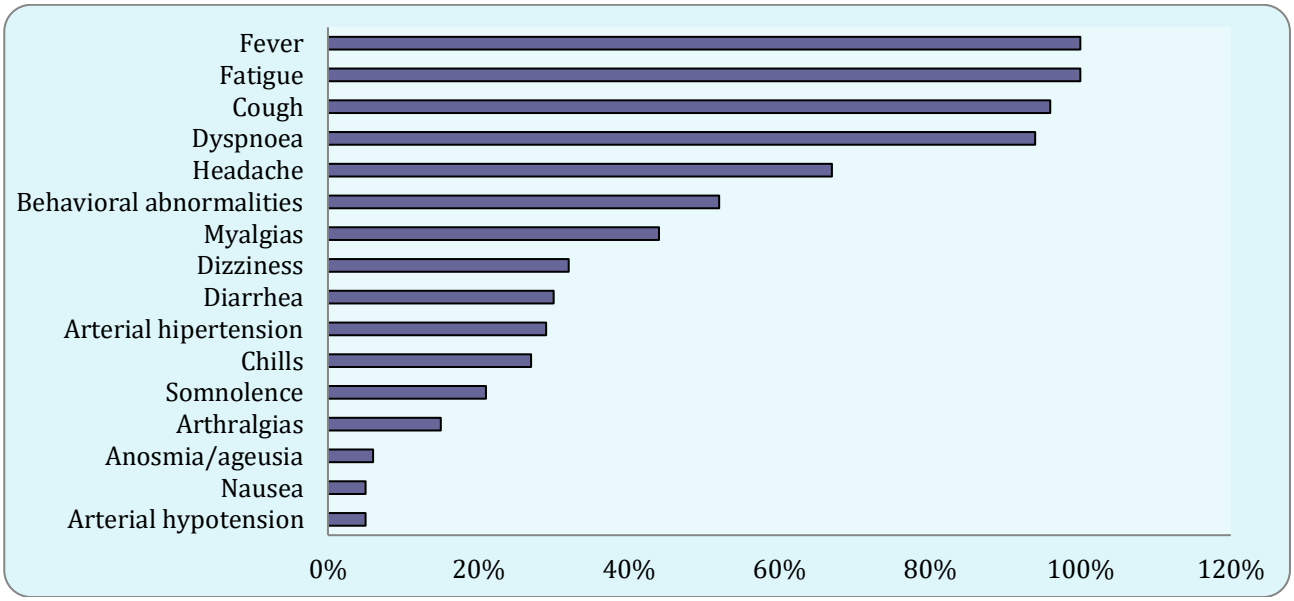


Figure 1. The main clinical manifestations in the total group while entrance to the hospital.

Patients also suffered from different comorbidities with negative potentiality on the evolution of COVID-19, such as arterial hypertension and chronic cardiovascular diseases 33 (50.0%) patients, diabetes mellites 21 (31.8%) patients, severe obesities 20 (30.3%) patients. Only 10 (15.15%) patients, average age 57.93±1.6 y.o., were without confirmed comorbidities at the moment of hospitalization. There wasn't a statistically significant difference in the L_1 and L_2 groups in the prevalence of comorbidities that could substantially influence the evolution of COVID-19 (tab. 1).

Table 1. Distribution of patients by study groups and main comorbidities.

Comorbidities	L_1 group TCZ 200 mg	L_2 group TCZ 400 mg	p	RR	95%CI (RR)	NNT
Arterial hypertension and chronic cardiovascular diseases	17 (51.5%)	16 (48.5%)	0.81	1.06	0.65-1.72	33.0
Diabetes mellites	9 (27.3%)	12 (36.4%)	0.43	0.75	0.37-1.53	11.0
Severe obesities	8 (24.2%)	12 (36.4%)	0.29	0.67	0.31-1.41	8.25
Chronic renal diseases	5 (15.1%)	5 (15.1%)	1.0	1.0	0.26-3.84	-
Chronic hepatitis	3 (9.1%)	4 (12.1%)	0.69	0.75	0.18-3.09	33.0
Chronic pulmonary diseases	1 (3.0%)	3 (9.1%)	0.32	0.32	0.04-2.96	16.3
Autoimmune thyroiditis	1 (3.0%)	3 (9.1%)	0.32	0.32	0.04-2.96	16.3

Note: RR and NNT were computed for the experimental group

The duration of fever before TCZ administration was 7.89±0.32 (1-14) days in the total group: L_1 group – 7.81±0.38 (3-13) days, L_2 group – 7.69±0.53 (1-14). This was on average on the 11.34±0.31 day of the disease, L_1 group – 11.36±0.44 (5-16), L_2 group – 11.33±0.43 (7-16), [95%CI (0.18-0.24), P=0.78].

The level of SpO_2 (%) before TCZ administration was 85.02±0.77 (68-94) in the total group, L_1 group – 85.09±1.03 (78-94), L_2 group – 84.94±1.15 (68-94), [95%CI (0,39-0,69), P=0,58]. It is necessary to mention that TCZ typically leads to the temporary worsening of the patients' general state within several days, usually manifested by the progression of asthenia and temporary reduction of SpO_2, usually by 5-7%. The first symptom of patient improvement was the decrease in the level of fever, then the increase of SpO_2, after that – asthenia reduction, and the last – dyspnea disappearance. Subsequently, administration of an IL-6 inhibitor, the improvement in the general state of the patients was TCZ-dose-dependent, with better results registered for the L_2 group, which also led to a shorter duration of oxygen therapy (tab. 2).

Table 2. Duration of clinical manifestations subsequently administration of TCZ.

Manifestations	L_1 group TCZ 200 mg	L_2 group TCZ 400 mg
Fever duration, days (minimum-maximum days)	2.09±0.25 (1-5)	0.28±0.09 * (1-2)
On what day post-TCZ the general state improves according to the patients' appreciation, (minimum-maximum days)	7.29±0.74 (1-8)	5.26±0.53 * (2-8)
Average of minimal SpO_2, % (minimum-maximum %)	88.7±0.64 (78-94)	90.15±0.6 * (80-94)
On what day does SpO_2 start to increase (minimum-maximum days)	4.95±0.46 (1-10)	4.08±0.46 * (1-14)
On what day does SpO_2 ≥95 (minimum-maximum days)	9.44±0.97 (3-22)	7.13±0.75 * (2-19)
Duration of asthenia, days (minimum-maximum days)	7.29±0.74 (2-16)	5.26±0.53 * (1-15)
Duration of oxygen therapy, days (minimum-maximum days)	10.9±0.94 (2-23)	7.87±0.75 * (1-19)
Duration of dyspnea, days (minimum-maximum days)	16.04 ±1.08 (6-29)	11.66±0.82 * (3-23)

Note: *p-significance coefficient (p< 0.0001)

At the same time, after TCZ administration 11 (33%) patients in L_1 group maintained fever for 1-5 days and 8 (24.2%) patients in L_2 group - for 1-2 days. Worsening of the general condition by intensification of dyspnea and general asthenia was established in 15 (45.5%) patients from L_1 group on average for 3.5 days and in 17 (51.5%) - from L_2 group for 2.8 days. Hypotension below 90/60 mmHg was recorded in 2 (6.0%) patients in L_1 group and in 7(21.2%) – in L_2 group. SpO_2 < 94% was maintained in 31 (93.9%) patients in L_1 group (during 3-22 days) and in 27 (81.8%) - in L_2 group (during 2-19 days)(tab.3).

The transfer rate to the ICU remains high in the total group, without statistically significant difference depending on the administration of TCZ dose. In the ICU department was transferred 18 (27.3%) patients from the total group, L_1 group – 8 (24.2%) patients, L_2 group – 10 (30.3%) patients, [95%CI (0.36-1.77), p=0.62]. According to the significance index "p", no statistically significant differences were established between the studied groups (tab. 3) although the relative risk of worsening after TCZ treatment (RR=0.88), relative risk of blood pressure drop (RR=0.29) and relative risk of transfer to intensive care unit (RR=0.8) were lower in L_1 patients compared to the control group. Based on the statistical indicators below, the TCZ 200 mg dose was not found to be less effective than the TCZ 400 mg dose. At the same time, the NNT is quite high according to all manifestations for the dose of TCZ 200mg compared to 400mg.

Table 3. Clinical status of patients after TCZ administration.

Manifestations	L_1 group TCZ 200 mg	L_2 group TCZ 400 mg	P	RR	95%CI (RR)	NNT
Presence of fever after TCZ treatment,patients	11	8	0.41	1.37	0.64-2.97	11.0
Post TCZ aggravation (dyspnea, asthenia), patients	15	17	0.46	0.88	0.54-1.45	11.0
Hypotension < 90/60 mmHg, patients	2	7	0.1	0.29	0.06-1.27	6.6
SpO_2 < 94%, patients	31	27	0.14	1.14	0.96-1.38	8.2
Transfer to intensive care wards, patients	8	10	0.62	0.80	0.36-1.77	16.5

Note: RR and NNT were computed for the experimental group

Radiological changes were registered in all patients from both groups before TCZ administration. The improvement of the radiological picture in L_1 patients was established on average 13.13±0.92 days after the administration of TCZ 200 mg, and in patients from the control group – at 11.33±0.83 days, significantly earlier (p≤0.0001).

The duration of hospitalization in non-transferred to ICU patients was on average 15.97±1.17 (8-30) days, statistically significantly longer in the L_1 group 17.5±1.53 (9-30), compared with the 2-nd group 14.5±1.69 (8-30), [95%CI (2.21-3.79), p≤0.0001].

DISCUSSIONS

In the Republic of Moldova, TCZ is included in the off-label treatment guidelines for COVID-19 for severe and critical cases. The usefulness of IL-6 inhibitors depends on the optimal time of administration: too early can inhibit the effectiveness of the host immune answer; too late – aggravate the clinical manifestation, leading to invasive ventilation in critically ill patients. That is why the clinical thinking of physicians, their knowledge proficiencies in understanding the COVID-19 evolution, and hypercytokinemia expectation, experience aptitudes in finding the proper time for TCZ administration is essential for the life of the patients.

We are confident of our study limitations, but despite these, we confirm that most baseline characteristics were similar between the two groups, and the main standard of management was identical. In our study, both doses of TCZ demonstrate its efficiency in severely ill patients with COVID-19, which is essential in a pandemic situation, especially during periods of TCZ supply shortages. We cannot prove that the dose of TCZ influences the percentage of patients transferred to the ICU for invasive or non-invasive ventilation. However, we demonstrate that the TCZ dose significantly impacts the duration of patients' disabilities, with better efficiency for TCZ 400 mg compared to 200 mg. That is why TCZ 200 mg may not be a cost-effective solution in COVID-19, as it leads to longer hospitalization and NNT is quite high according to all manifestations than TCZ 400 mg. However, reducing the TCZ dose can be a life-saving option in the context of ongoing supply shortages.

CONCLUSIONS

1. The improvement of general condition by decreasing febrile period, asthenia, disappearance of dyspnea and increase of SpO_2 level, improvement of radiological picture, as well as duration of hospitalization showed better results in patients treated with TCZ 400 mg, although the relative risk of rapid worsening after TCZ treatment (RR=0.88), relative risk of blood pressure decrease (RR=0.29) and relative risk of transfer to intensive care unit for invasive or non-invasive ventilation (RR=0.8) were lower in patients treated with TCZ 200 mg compared to the TCZ 400 mg.
2. The 200 mg fixed dose of Tocilizumab may be considered an alternative and life-saving drug for severely ill patients at the beginning of the hypercytokinemia phase of COVID-19 in the context of IL-6 inhibitor supply deficiency can provide benefits to a larger number of patients.
3. Further randomized trials are needed to confirm the efficacy of a low dose of Tocilizumab and the frame time of IL-6 inhibitor administration in larger populations.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

ACKNOWLEDGMENT

There is no information.

ETHICAL APPROVAL

This study was carried out with the approval of the Ethics Committee no. 02/02.10.2020 of the *Nicolae Testemitanu* State University of Medicine and Pharmacy, Republic of Moldova.

REFERENCES

1. Kany S, Vollrath JT, Relja B. Cytokines in Inflammatory Disease. *Int J Mol Sci.* 2019; 20(23):6008. doi:10.3390/ijms20236008
2. Zhang JM, An J. Cytokines, inflammation, and pain. *Int Anesthesiol Clin.* 2007;45(2):27-37. doi:10.1097/AIA.0b013e318034194e
3. Rabaan AA, Al-Ahmed SH, Muhammad J, et al. Role of Inflammatory Cytokines in COVID-19 Patients: A Review on Molecular Mechanisms, Immune Functions, Immunopathology, and Immunomodulatory Drugs to Counter Cytokine Storm. *Vaccines (Basel).* 2021;9(5):436. doi:10.3390/vaccines9050436
4. Jamilloux Y, Henry T, Belot A, et al. Should we stimulate or suppress immune responses in

COVID-19? Cytokine and anti-cytokine interventions. *Autoimmun Rev.* 2020;19(7): 102567. doi:10.1016/j.autrev.2020.102567
5. Giamarellos-Bourboulis EJ, Netea MG, Rovina N, et al. Complex immune dysregulation in COVID-19 patients with severe respiratory failure. *Cell Host Microbe.* 2020. doi:10.1016/j. chom.2020.04.009
6. Siddiqi HK, Mehra MR. COVID-19 illness in native and immunosuppressed states: A clinical-therapeutic staging proposal. *J Heart Lung Transplant.* 2020;39(5):405-407. doi:10.1016/j.healun.2020.03.012
7. Dadras O, Afsahi AM, Pashaei Z, et al. The relationship between COVID-19 viral load and disease severity: A systematic review. *Immun Inflamm Dis.* 2022;10:e580. doi:10.1002/iid3.580
8. Shekhawat J, Gauba K, Gupta S, et al. Interleukin-6 Perpetrator of the COVID-19 Cytokine Storm. *Indian J Clin Biochem.* 2021; 36(4):1-11. doi:10.1007/s12291-021-00989-8
9. Santa Cruz A, Mendes-Frias A, Oliveira A, et al. Interleukin-6 Is a Biomarker for the Development of Fatal Severe Acute Respiratory Syndrome Coronavirus 2 Pneumonia. *Front. Immunol.* 12:613422. doi:10.3389/fimmu.2021.613422
10. McGonagle D, Sharif K, O'Regan A, Bridgewood C. The Role of Cytokines including Interleukin-6 in COVID-19 induced Pneumonia and Macrophage Activation Syndrome-Like Disease. *Autoimmun Rev.* 2020;19(6): 102537. doi:10.1016/j.autrev.2020.102537
11. Zhang Y, Zhang Z, Wei R, et al. IL (Interleukin)-6 Contributes to Deep Vein Thrombosis and Is Negatively Regulated by miR-338-5p. *Arterioscler Thromb Vasc Biol.* 2020;40(2): 323-334. doi:10.1161/ATVBAHA.119.313137
12. Kerr R, Stirling D, Ludlam Ch. Interleukin 6 and Haemostasis. *BJHaem.* 2008. doi:10.1046/j.1365-2141.2001.03061
13. Pons S, Fodil S, Azoulay E. et al. The vascular endothelium: the cornerstone of organ dysfunction in severe SARS-CoV-2 infection. *Crit Care 24.* 2020. doi:10.1186/s13054-020-03062-7
14. Leisman DE, Deutschman CS, Legrand M. Facing COVID-19 in the ICU: vascular dys function, thrombosis, and dysregulated inflammation. *Intensive Care Med.* 2020;46(6): 1105-1108. doi:10.1007/s00134-020-06059-6
15. Stenmark KR, Frid MG, Gerasimovskaya E, et al. Mechanisms of SARS-CoV-2-induced lung vascular disease: potential role of complement. *Pulm Circ.* 2021;11(2): 20458940211015799. doi:10.1177/20458940211015799
16. Xu X, Han M, Li T, Sun W, et al. Effective treatment of severe COVID-19 patients with tocilizumab. *Proc Natl Acad Sci USA.* 2020; 117(20):10970-10975. doi:10.1073/pnas.2005615117
17. San-Juan R, Fernández-Ruiz M, López-Medrano F, et al. Analysis of the factors predicting clinical response to tocilizumab therapy in patients with severe COVID-19. *Int J Infect Dis.* 2022. doi:10.1016/j.ijid.2022.01.040
18. Roumier M, Paule R, Vallée A, et al. Tocilizumab for Severe Worsening COVID-19 Pneumonia: a Propensity Score Analysis. *J Clin Immunol.* 2021;41(2):303-314.
19. Somers E, Eschenauer G, Troost J, et al. Tocilizumab for Treatment of Mechanically Ventilated Patients With COVID-19. *Clinical Infectious Diseases.* 2021;73(2):e445-e454. doi:10.1093/cid/ciaa954
20. Chamber D, Aksak-Wąs B, Bobrek-Lesiakowska K, et al. Effectiveness of Tocilizumab in Patients with Severe or Critical Lung Involvement in COVID-19: A Retrospective Study. *J Clin Med.* 2022;11(9):2286. doi:10.3390/jcm11092286
21. RECOVERY Collaborative Group. Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. *The Lancet.* 2021;397. doi:10.1016/S0140-6736(21)00676-0
22. Gordon AC, Mouncey PR, Al-Beidh F, et al. Interleukin-6 receptor antagonists in critically ill patients with COVID-19. *N Engl J Med.* 2021;384:1491-1502. doi:10.1056/NEJMoa2100433
23. WHO. A living WHO guideline on drugs for covid-19. *BMJ.* 2020;370:m3379.
24. NIH Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. Available from: https://www.covid19treatmentguidelines.nih.gov/ [Accessed 02.06.2022].

25. IDSA. Guidelines on the Treatment and Management of Patients with COVID-19. Available from: https://www.idsociety.org/practice-guideline/covid-19-guideline-treatment-and-management/ [Accessed 02.06.2022].
26. MSMPS. Infectia cu coronavirus de tip nou (COVID-19) [New type of coronavirus infection (COVID-19)], PCN 371. Available from: https://ms.gov.md/legislatie/covid-19/protocoale-si-ghiduri-covid-19/ [Accessed 02.06.2022].
27. Ercan S, Ergan B, Özuygur S, Korkmaz P, et al. Clinical Predictors of Response to Tocilizumab: A Retrospective Multicenter Study. *Turk Thorac J.* 2022;23(3):225-230. doi:10.5152/TurkThoracJ.2022.21179
28. De Rossi N, Scarpazza C, Filippini Ch. Early use of low dose tocilizumab in patients with COVID-19: A retrospective cohort study with a complete follow-up. *EClinicalMedicine*. 2020;25:100459. doi:10.1016/j.eclinm.2020.100459
29. Hermine O, Mariette X, Porcher R, et al. Effect of Interleukin-6 Receptor Antagonists in Critically Ill Adult Patients with COVID-19 Pneumonia: two Randomised Controlled Trials of the CORIMUNO-19 Collaborative Group. *European Respiratory Journal.* 2022;2102523. doi:10.1183/13993003.02523-2021
30. Cardona-Pascual I, Berlana D, Martinez-Valle F, et al. Effect of tocilizumab versus standard of care in adults hospitalized with moderate-severe COVID-19 pneumonia. *Med Clin (Engl Ed).* 2022;158(7):301-307. doi:10.1016/j.medcle.2021.03.036
31. Rosas I, Brau N, Waters M, et al. Tocilizumab in patients hospitalised with COVID-19 pneumonia: Efficacy, safety, viral clearance, and antibody response from a randomised controlled trial (COVACTA). *eClinicalMedicine.* 2022;47:101409. doi:10.1016/j.eclinm.2022.101409
32. Stukas S, Joshua G, Kinkade A, et al. Reduced fixed dose tocilizumab 400 mg IV compared to weight-based dosing in critically ill patients with COVID-19: A before-after cohort study. *Lancet Reg Health Am.* 2022;11:100228. doi:10.1016/j.lana.2022.100228

Date of receipt of the manuscript: 04/08/2022
Date of acceptance for publication: 07/07/2023

Stela COJOCARU, SCOPUS ID: 57404918900; WoS Researcher ID: AEB-7845-2022
Irina RUSSU, WoS Researcher ID: AAE-2463-2022
Galina BUTA, SCOPUS ID: 57405570900

OH&RM ONE HEALTH & RISK MANAGEMENT

VOL. 4, ISSUE 4
2023

BIOLOGICAL SCIENCES

OPEN ACCESS

ISOLATION, IDENTIFICATION, AND CONSERVATION OF BIOTECHNOLOGICALLY RELEVANT BACTERIA FROM THE WATER OF "LA IZVOR" LAKE

Ludmila BALAN (BATIR), Valerina SLANINA, Nina BOGDAN-GOLUBI

The Institute of Microbiology and Biotechnology of Technical University of Moldova, Chisinau, Republic of Moldova

Corresponding author: Ludmila Balan, e-mail: ludmila.batir@imb.utm.md

DOI: 10.38045/ohrm.2023.4.03 CZU: 579.68:556.55(478-25)

Keywords: *microbial biodiversity, isolation, molecular identification, long-term conservation, sustainable agriculture.*

Introduction. *The identification of biotechnologically relevant bacterial strains allows for their utilization as a basis for the development of sustainable agriculture technologies.* ***Material and methods.*** *From the lakes of "La Izvor" Park, 213 representatives of various genera were isolated, out of which 148 were pathogenic. Each suspension culture was diluted until 10^{-8}, serial dilutions were inoculated on plates with Tryptic Soy Agar, Kings medium B, Salmonella Shigella Agar, Kligler's Iron Agar, Endo agar. For the non-pathogenic ones, enzymatic properties were determined, antimicrobial activity was assessed, and they were identified using the Polymerase Chain Reaction (PCR) method. Selected strains were preserved through lyophilization for an extended period.* ***Results.*** *The study revealed significant microbial biodiversity in the lakes of "La Izvor" Park, from which 213 bacterial strains were isolated in pure culture, with 65 selected for further research. The purpose of the research was to isolate and identify non-pathogenic biotechnologically relevant bacteria with various properties that could be applied in the development of sustainable agriculture. Nineteen strains with enhanced enzymatic properties and high antifungal activity were highlighted. These strains were molecularly identified and deposited for inclusion in the National Collection of Non-pathogenic Microorganisms (CNMN).* ***Conclusions.*** *The results of our study have shown that microorganisms can be used as safe biological control agents without the use of chemical compounds, which can prevent soil and water contamination. Lyophilization has confirmed that these strains have tolerance to osmotic and thermal shocks while maintaining viability of over 75%. Based on these properties, the strains are ideal candidates for application in sustainable agriculture.*

Cuvinte-cheie:*. biodiversitate microbiană, izolare, identificare moleculară, conservare îndelungată, agricultură durabilă.*

IZOLAREA, IDENTIFICAREA ŞI CONSERVAREA BACTERIILOR DE INTERES BIOTEHNOLOGIC DIN APA LACURILOR DIN PARCUL „LA IZVOR"

Introducere. *Identificarea tulpinilor de bacterii de interes biotehnologic permite valorificarea lor ca suport pentru dezvoltarea tehnologiilor agriculturii durabile.* ***Material şi metode.*** *Din lacurile parcului „La Izvor" au fost izolaţi 213 reprezentanţi ai diferitor genuri, dintre care – 148 de tulpine patogene. La fiecare cultură a fost obţinută o serie de diluţii zecimale până la 10^{-8}, cu inoculare pe medii agarizate Tryptic Soy Agar, Kings medium B, Salmonella Shigella Agar, Kligler's Iron Agar, Endo agar. La cele nepatogene au fost determinate proprietăţile enzimatice, activitatea antimicrobiană, au fost identificate prin metoda Polymerase Chain Reaction (PCR) şi tulpini selectate au fost conservate prin liofilizare pentru o perioadă îndelungată.* ***Rezultate.*** *Studiul a relevat o biodiversitate microbiană semnificativă în lacurile parcului „La Izvor", de unde au fost izolate în cultură pură 213 tulpini bacteriene, dintre care 65 au fost selectate pentru cercetări ulterioare. Scopul studiului a constat în izolarea şi identificarea bacteriilor nepatogene de interes biotehnologic, cu diferite proprietăţi, care ar putea fi aplicate în dezvoltarea agriculturii durabile. Au fost evidenţiate 19 tulpini cu proprietăţi enzimatice sporite şi cu activitate antifungică înaltă. Aceste tulpini au fost identificate prin metode biologice moleculare şi au fost depuse pentru completarea Colecţiei Naţionale de Microorganisme Nepatogene (CNMN).*

Concluzii. *Rezultatele studiului nostru au relevat că microorganismele pot fi utilizate ca agenţi de control biologic de siguranţă, fără utilizarea compuşilor chimici, în acest mod prevenindu-se contaminarea solurilor şi a apelor. Liofilizarea a confirmat că aceste tulpini au toleranţă la şocurile osmotice şi termice, cu păstrarea viabilităţii de peste 75%. Conform acestor proprietăţi, tulpinile sunt candidaţi ideali pentru aplicare în agricultura durabilă.*

INTRODUCTION

Conserving biodiversity involves maintaining, increasing, or actively managing the abundance and variety of all species worldwide, regardless of classification, ecosystems, or genetic diversity (1, 2, 3).

Biodiversity is defined as the variety of all life on Earth: ecosystems and their living organisms, including animals, plants, habitats, microorganisms, and their genes. Biodiversity assessment is the first step in the process of defining biodiversity management objectives and conservation measures for a particular area. The goal of assessment is to gather, analyse, and evaluate the most relevant information to inform decision-making and provide recommendations for the future (1, 2, 3).

For the conservation of biodiversity, the focus is often placed on the most important species, which can be grouped based on certain indicators, as biodiversity holds multifaceted significance for humans. It plays a crucial role in our lives because we depend on the products it provides (3).

Various species with their characteristics and roles form one of the most important foundations for sustaining human life. It's important to remember that, although their role has not yet been scientifically proven, each species is important and has a well-defined role in the Earth's web of life. Microorganisms, due to their rich and diverse enzymatic equipment, can metabolize a wide variety of organic and inorganic compounds (1, 2, 3). Biodiversity conservation can be *ex-situ* (outside their natural habitats, as mentioned in Article 9 of the CBD) or *in-situ*, referring to the protection of species in their natural habitats (3).

Ex-situ conservation involves methods for removing individuals of various species from their natural environment for purposes such as reproduction, storage, cloning, or rescue, especially in cases where their habitats can no longer support their existence or when they can be used for research and public awareness materials. The purpose of *ex-situ* conservation of species is diverse and includes scientific research activities, the production of individuals for *in-situ* reintroduction programs, the maintenance of genetic diversity, and the establishment of self-sustaining captive populations (auto-conservation), so that no individuals of the species are taken from the wild.

Thus, *ex-situ* conservation programs should be considered as a last resort (1, 2, 3).

Within the National Collection of Non-pathogenic Microorganisms (CNMN), the method of lyophilization is widely used for the long-term conservation and preservation of microorganisms. Lyophilization is a common and safe method for maintaining the biological properties of microorganisms. It allows for the long-term maintenance of cultures without the need for continuous subculturing, which is a clear advantage, as repeated subculturing can lead to changes in biological properties. To date, there has been extensive experience in applying the lyophilization method for preserving microbial cultures. The successful preservation in a dry state relies on strict adherence to all the details of the lyophilization process and the conditions for storing lyophilized cultures. Maintaining the viability and purity of strains while preserving their valuable properties is an important task for every microbial collection, from scientific research to practical implementation (4 – 8).

Our research has focused on isolating, identifying, and conserving aquatic microbial biodiversity from the "La Izvor" lakes in Chisinau municipality. The aim was to select biotechnologically relevant strains based on their enzymatic and antimicrobial properties to contribute to the development of sustainable agricultural and technological advancements (9).

To achieve this *aim*, a screening of aquatic biodiversity was conducted, leading to the isolation of various species of microorganisms such as bacteria (including cyanobacteria and actinobacteria), fungi (both mycelial and yeast), and microalgae. Among all the groups of isolated microorganisms, the study of bacterial strain diversity is of particular interest, as they represent a wide variety and can be both pathogenic and non-pathogenic.

MATERIAL AND METHODS

The study included 213 types of bacteria isolated as a result of assessing the aquatic biodiversity from the water, mud, and biofilms formed on the lakes in "La Izvor" Park. The samples were collected from various sectors within the park.

For the isolation, cultivation, and subculturing of bacterial cultures, the following culture media

were used: nutrient agar, agarized meat peptone medium, meat peptone broth, King A, and King B (dilutions 10^{-5} and 10^{-6}) (10, 11, 12). The isolation of bacterial cultures was carried out through successive dilutions. Subsequently, as a result of these successive dilutions, they were inoculated onto Petri dishes with various culture media.

To identify strains of pathogenic enterobacteria, the following culture media were used: Salmonella Shigella Agar, Endo Agar, Kligler Iron Agar (KIA), and Triple Sugar Iron Agar (TSI) (13 – 16).

For species identification, molecular biological methods were employed, involving the amplification and sequencing of the 16S rRNA (17).

For conservation, through the lyophilization method, 19 strains of biotechnologically relevant bacteria were selected based on their high antimicrobial properties against mycelial fungi and phytopathogenic bacteria. These strains are now stored in the National Collection of Non-pathogenic Microorganisms (CNMN) at the Institute of Microbiology and Biotechnology of the Technical University of Moldova: *Bacillus velezensis* CNMN-BB-12, *Bacillus velezensis* CNMN-BB-13, *Bacillus velezensis* CNMN-BB-14, *Bacillus velezensis* CNMN-BB-15, *Bacillus velezensis* CNMN-BB-16, *Bacillus velezensis* CNMN-BB-17, *Bacillus velezensis* CNMN-BB-18, *Micrococcus yunnanensis* CNMN-BM-19, *Micrococcus yunnanensis* CNMN-BM-20, *Paenibacillus pabuli* CNMN-BP-21, *Planococcus ruber* CNMN-BP-22, *Peribacillus simplex* CNMN-BP-23, *Planococcus chinensis* CNMN-BP-24, *Bacillus safensis* CNMN-BB-25, *Bacillus safensis* CNMN-BB-26, *Bacillus safensis* CNMN-BB-27, *Peribacillus simplex* CNMN-BP-28, *Bacillus rugosus* CNMN-BB-29, *Micrococcus aloeverae* CNMN-BM-30.

As a nutritional medium for the cultivation and growth of the mentioned strains, nutrient agar was used. Cultivation was carried out at a temperature of +30±1°C for a period of 24-48 hours.

As a stabilizing medium with a protective effect against osmotic and thermal shocks for lyophilization, 7% sucrose in skim milk was used. After resuspending the strains in the protective medium and distributing them into vials with 1 mL each, the samples were frozen at -80°C in the *ARCTICO ULTF 80* freezer, then lyophilized using the *Free Zone Plus* lyophilized. After lyophilization, they were sealed and stored at +4°C.

The subculturing of microorganism cultures after lyophilization was carried out on nutrient agar medium, following the standard method. This involved rehydrating them with distilled water and incubating them at a temperature of +30±1°C for 3 hours to facilitate their revival (10, 11, 12, 18).

The determination of the viability of microorganism cultures was performed using the method proposed by Donev in 2002 (1). The method involves performing successive dilutions, inoculating onto Petri dishes, and counting the colony-forming units, following a specific formula:

$$c\ \% = (\lg UFC ml^{-1}_{fin} / \lg UFC ml^{-1}_{in}) \times 100\%$$

in which:

lgUFC ml^{-1}_{in} represents the logarithm with base 10 of the number of colony-forming units before lyophilization;
lgUFC ml^{-1}_{fin} represents the logarithm of the number of colony-forming units after lyophilization or storage;
c – the viability of cultures in percentages.

Statistical analysis was performed using Microsoft Excel 2010, by logarithmizing the data and determining the percentage of viability. The statistical significance threshold was set at p=0.05.

RESULTS

In order to study the aquatic biodiversity of the lakes in "La Izvor" Park, 11 water samples, 11 mud samples, and 11 biofilms were examined. From these samples, 213 types of bacterial colonies were isolated. Subsequently, for the purpose of selecting pathogenic isolates of enterobacteriaceae, they were cultivated on characteristic media including SS agar, Endo, KIA, and TSI. As a result of the analysis of bacterial growth on these characteristic media, 148 samples of enteropathogenic bacteria were identified, including Escherichia coli, Salmonella, Shigella, Enterobacter, Klebsiella, and other strains from the Enterobacteriaceae family (9).

The remaining 65 isolates in the study were tested for various parameters to identify the most active ones, which are biotechnologically relevant to us. Consequently, enzymatic properties such as amylolytic activity (detected in 27 strains), catalase activity (in 42 strains), lipase activity (in 21 strains), cellulase activity (in 18 strains) were determined. Additionally, their antifungal and antibacterial activities were assessed. As a result of

the screening, the most active 19 strains were selected. These selected strains were later identified using molecular biological methods (amplification and sequencing of 16S rRNA) in collaboration with the Institute of Biology in Bucharest, Romania, and the Mother and Child Institute in Chisinau, Moldova (17).

Using molecular biology techniques, it was determined that the majority of the isolated strains were identified as *Bacillus velezensis*, with 16S rRNA sequences showing a similarity of over 99.6% (and a 99.4% similarity with *Bacillus amyloliquefaciens* MPA 1034). Additionally, strains of *Micrococcus yunnanensis* were identified, where the 16S rRNA sequences exhibited a similarity of over 99.7% (and a 99.5% similarity with *Micrococcus luteus* DSM 200030) (17).

Following microscopic examination, the selected bacterial strains are both Gram-positive and Gram-negative. On nutrient agar medium, they form colonies that can be white or yellow, matte or shiny, with a smooth and wavy edge. Some may be of the "S" type, while others are "R." Microscopically, the cells have a spherical cocci shape and do not form spores (as seen in *M. yunnanensis, Planococcus ruber/Planococcus massiliensis*), while others have an elongated rod-shaped (bacilli) form (as seen in *B. velezensis, Paenibacillus pabuli*), which can form endospores (9).

The selected strains with biotechnological potential were described according to the deposition passport at CNMN and deposited with the issuance of a microorganism storage certificate. After deposition at CNMN, these microorganism strains are preserved using various methods: through periodic subculturing (though this method is not efficient for long-term preservation, as there is a risk of culture loss or changes in the initial morpho-cultural characteristics), under a layer of mineral oil, or by lyophilization (which is the safest method) (4, 5, 6, 19). Thus, as a result of preserving bacterial strains through the lyophilization method, it can be observed that the cell viability after lyophilization varies between 75.91% to 93.08%, depending on the studied strain (tab. 1).

The analysis of the data in the table above also shows that, for some strains, the titer decreases by 3 units compared to the data prior to lyophilization, while for others, it only decreases by 1 unit. This could be associated with their resistance to the osmotic and thermal shocks they were subjected to. Thus, for those with a sudden drop in titer of up to 3 units, the post-lyophilization viability is lower and ranges between 75.91% and 79.84%. Meanwhile, for those where the titer drops by one unit, the viability is higher, ranging between 85.17% and 93.08%. The highest viability, reaching 93.08%, is observed in the *Bacillus velezensis* CNMN-BB-16 and *Peribacillus simplex* CNMN-BP-23 strains.

All bacterial strains revived from lyophilized state have achieved viability values of over 75.91%, indicating a very good index for cultures preserved for 5 to 19 years. The results obtained confirm the preservation of biotechnological properties, morpho-cultural characteristics, and the enhanced regenerative capacity of the described strains, as observed by other researchers (4, 5, 20).

DISCUSSIONS

The conservation of biological diversity at the level of ecosystems, species, populations, and genes is one of the main concerns of humanity in the 21st century (the third millennium). The problem lies in the fact that, with the advancement of technological progress and the intensive use of natural resources, the anthropogenic impact on biological diversity has significantly increased, leading to a substantial reduction in the number of species and varieties of living organisms that inhabit the Earth (1, 2, 3).

To identify microorganisms down to the genus or species level, a series of tests are necessary. These tests include both morphological and cultural characteristics, as well as a series of biochemical tests for microorganism determination. This is because microorganisms utilize various chemical substances from the culture medium as a source of energy or as building material for growth and reproduction, resulting in changes to the initial environment (the disappearance of certain substances from the medium, the appearance of new products, alterations in pH, gas production, etc.) (21, 22). These modifications reflect the nature of the enzymatic equipment and, indirectly, the genetic characteristics of the respective microorganisms. Therefore, biochemical properties are of particular importance for the identification and classification of microorganisms. Some characteristic biochemical tests include agglutination tests, catalase tests, oxidase tests, coagulation tests, and others.

Table 1. The viability of bacterial strains isolated from the water of "La Izvor" Lake, both before and after lyophilization.

No	Strain name	Colony-Forming Units (CFU), mL-1		Viability, %
		Before lyophilization	After lyophilization	
1	***Bacillus velezensis* CNMN-BB-12**	$1.2 \times 10^{12} \pm 0.1$	$2.3 \times 10^{9} \pm 0.4$	77.41±0.7
2	***Bacillus velezensis* CNMN-BB-13**	$1.8 \times 10^{12} \pm 0.2$	$3.9 \times 10^{9} \pm 0.2$	78.31±0.3
3	***Bacillus velezensis* CNMN-BB-14**	$9.3 \times 10^{11} \pm 0.7$	$1.2 \times 10^{9} \pm 0.3$	75.91±1.0
4	***Bacillus velezensis* CNMN-BB-15**	$1.7 \times 10^{12} \pm 0.2$	$2.9 \times 10^{9} \pm 0.5$	77.31±0.5
5	***Bacillus velezensis* CNMN-BB-16**	**$7.3 \times 10^{8} \pm 1.7$**	**$1.8 \times 10^{8} \pm 0.2$**	**93.08±0.6**
6	***Bacillus velezensis* CNMN-BB-17**	$1.1 \times 10^{12} \pm 0.1$	$1.6 \times 10^{9} \pm 0.3$	76.39±0.9
7	***Bacillus velezensis* CNMN-BB-18**	$1.3 \times 10^{12} \pm 0.1$	$1.8 \times 10^{9} \pm 0.3$	76.52±0.5
8	***Micrococcus yunnanensis* CNMN-BM-19**	$1.5 \times 10^{12} \pm 0.1$	$2.5 \times 10^{9} \pm 0.5$	76.05±0.9
9	***Micrococcus yunnanensis* CNMN-BM-20**	$1.8 \times 10^{12} \pm 0.1$	$3.1 \times 10^{9} \pm 0.5$	76.33±0.3
10	***Paenibacillus pabuli* CNMN-BP-21**	$2.2 \times 10^{12} \pm 0.1$	$7.2 \times 10^{9} \pm 0.4$	79.84±0.3
11	***Planococcus ruber* CNMN-BP-22**	$2.1 \times 10^{12} \pm 0.1$	$6.3 \times 10^{9} \pm 0.7$	79.51±0.4
12	***Peribacillus simplex* CNMN-BP-23**	**$1.5 \times 10^{9} \pm 0.2$**	**$3.5 \times 10^{8} \pm 1.1$**	**93.08±1.9**
13	***Planococcus chinensis* CNMN-BP-24**	$1.4 \times 10^{9} \pm 0.1$	$1.6 \times 10^{8} \pm 0.3$	89.75±0.7
14	***Bacillus safensis* CNMN-BB-25**	$8.8 \times 10^{9} \pm 0,7$	$5.1 \times 10^{8} \pm 0.3$	87.59±0.6
15	***Bacillus safensis* CNMN-BB-26**	$9.2 \times 10^{9} \pm 0.4$	$6.2 \times 10^{8} \pm 0.4$	88.23±0.1
16	***Bacillus safensis* CNMN-BB-27**	$1.1 \times 10^{10} \pm 0.02$	$7.6 \times 10^{8} \pm 1.0$	88.56±0.6
17	***Peribacillus simplex* CNMN-BP-28**	$6.5 \times 10^{9} \pm 0.8$	$2.3 \times 10^{8} \pm 0.5$	85.17±1.2
18	***Bacillus rugosus* CNMN-BB-29**	$1.3 \times 10^{10} \pm 0.1$	$1.0 \times 10^{9} \pm 0.01$	89.13±0.2
19	***Micrococcus aloeverae* CNMN-BM-30**	$1.2 \times 10^{10} \pm 0.1$	$1.1 \times 10^{9} \pm 0.1$	89.90±0.2

**p=0,05*

For the selection of pathogenic isolates of Enterobacteriaceae, seeding on Endo agar allows for the isolation and identification of *Escherichia coli* strains. These will manifest as colonies with a dark pink to light red color, displaying a greenish metallic sheen, and the medium may exhibit marked reddening. On SS Agar, according to the data from specialized literature, *Salmonella* will not ferment lactose but will produce hydrogen sulfide (H_2S), thus the bacterial colonies formed will appear colorless with black centers. *Shigella* strains neither ferment lactose nor produce H_2S, resulting in colorless colonies as well (13 – 16).

Coliform bacteria, such as *E. coli*, can also be differentiated in this medium. E. coli will ferment lactose in the medium, resulting in bacterial growth with a pink color and no hydrogen sulfide production. *Enterobacter* and *Klebsiella* typically appear larger than *E. coli*, with colonies that are creamy mucoid, pale, opaque to pink (13, 14).

Another specific medium used for the isolation and selection of pathogenic cultures is KIA and TSI medium, which are widely employed in identifying Gram-negative bacteria, particularly from the *Enterobacteriaceae* family. These media are identical except for the fact that TSI contains an overdose of dextrose and lactose compared to what is found in KIA (13 – 16). The media are poured into slant tubes and inoculated with a stab followed by a streak on the slant surface. As a result, bacteria are exposed to both anaerobic conditions (at the bottom) and aerobic conditions (on the slant). Phenol red is present as an indicator. If the bacteria are non-fermentative, such as *Pseudomonas*, they can grow on slant tubes by aerobic degradation of proteinaceous components in the medium. This test is particularly valuable in the initial identification of the *Enterobacteriaceae* family (13 – 16).

For fermentative organisms, glucose is the first catabolized sugar. If glucose is consumed, the bacteria take a detour in their metabolic pathway. However, if glucose is limited, and the organism does not produce the necessary enzymes to catabolize lactose, the organism can use the protein from the medium. After inoculation on these given media, the culture is incubated for 24 hours at a temperature of +37°C. Time is crucial in reading the KIA results. An early reading could reveal yellow throughout the medium, leading to the conclusion that the organism is a lactose fermenter when it may simply not have exhausted the glucose yet. A reading after lactose has been exhausted could mean that the organism may be just a glucose fermenter (13, 14, 15).

Thus, the practical importance of biochemical properties has led to a diversification of working techniques and, in some cases, their standardization. Biochemical tests are performed only with the help of pure cultures, using control cultures that produce positive and negative reactions (18, 23).

The author Huda isolated and characterized the carotenoid pigment from *Micrococcus luteus*, which exhibited antibacterial and antifungal activity, along with the ability to absorb UV rays in the range of 300 to 500 nm (24). It is well known that aerobic bacteria of the *Bacillus* genus exhibit high antagonistic activity (22), and biological preparations based on them offer significant advantages, such as safety for humans and animals, resistance to adverse environmental factors. This makes them effective for use against plant diseases caused by phytopathogens (5, 19, 25). Devi S. and colleagues mentioned that *B. velezensis* FZB42 is the most extensively researched biofertilizer and biocontrol agent based on Bacillus, commercially used in agriculture (26). Moreover, researchers Li-Ting Wang and Fan have proposed a heterotypic synonym for *Bacillus velezensis*, with the subsequent name *Bacillus amyloliquefaciens*. This proposal has been confirmed through conducted studies and may be considered for further research (27, 28, 29).

The bacterial species *Bacillus amyloliquefaciens, Bacillus siamensis, Bacillus velezensis*, and *Bacillus nakamurai*, belonging to the operational group *Bacillus amyloliquefaciens* (OGBa), are all Gram-positive, spore-forming organisms. They are widely distributed in various niches, including soil, plants, food, and water. Members of the OGBa group are known as Plant Growth-Promoting Bacteria (PGPB) due to their abilities to fix nitrogen, solubilize phosphate, produce siderophores, phytohormones, antimicrobial compounds, and various enzymes (such as amylase, protease, lipase, cellulase, xylanase, pectinase, aminotransferase, barnase, peroxidase) (27, 28).

After identifying and determining strains of biotechnologically relevant bacteria, their conservation is of paramount importance due to the long-term preservation of their characteristic morphocultural and biochemical properties. The determination of the number of microbial cells immediately after lyophilization is a crucial parameter, allowing us to estimate the viability of the studied strains. A high number of viable cells after lyophilization can ensure the preservation of strain viability for an extended period. During the storage period, the viability of lyophilized cells tends to decrease relatively slowly (4, 6).

CONCLUSIONS

1. 213 types of bacterial colonies were isolated from the lakes in “La Izvor” Park, out of which 148 were enteropathogenic, such as *Escherichia coli, Salmonella, Shigella, Enterobacter*, and *Klebsiella*, as well as other strains from the *Enterobacteriaceae* family.
2. Nineteen strains of bacteria with enhanced enzymatic and antifungal properties were identified. Subsequently, they were characterized through molecular biological methods, described morphologically, and stored in the National Collection of Non-pathogenic Microorganisms.
3. The selected strains of biotechnologically relevant bacteria were preserved using the lyophilization method, which enabled a viability of over 75.91%. This high viability ensures their long-term preservation.
4. The strains *Bacillus velezensis* CNMN-BB-16 and *Peribacillus simplex* CNMN-BP-23 demonstrated remarkable resilience to the stresses they were subjected to, achieving the highest viability, reaching up to 93.08%.

CONFLICT OF INTEREST

There are no conflicts of interest.

FUNDING STATEMENT AND ACKNOWLEDGEMENTS

The research was conducted under the project 20.80009.7007.09, which was funded by the National Agency for Research and Development.

We offer our sincere thanks to our colleagues at the Institute of Biology in Bucharest, Madalin Iancu ENACHE, Robert RUGINESCU, and to those at the IMSP Mother and Child Institute, Victoria SACARA and Alexandr DORIF, who contributed to the identification of bacterial strains through molecular biological methods.

ETHICAL APPROVAL

The article does not have ethical approval.

REFERENCES

1. Ministerul Mediului Republicii Moldova. Strategia privind diversitatea biologică a Republicii Moldova pentru anii 2015-2020 şi Planul de acţiuni pentru implementarea acesteia. Hotărârea Guvernului Nr. 274 din 18-05-2015 [Ministry of the Environment of the Republic of Moldova. The strategy regarding the biological diversity of the Republic of Moldova for the years 2015-2020 and the action plan for its implementation. Government Decision No. 274 of 18-05-2015]. Available from: https://www.legis.md/cautare/getResults?doc_id=66444&lang=ro [Accessed 02 May 2015].
2. World Economic Forum. The Global Risks Report 2020. Available from: https://www3.weforum.org/docs/WEF_Global_Risk_Report_2020.pdf [Accessed 02 May 2015].
3. UNDP-GEF/MSP Republica Moldova. *Ghid pentru managementul conservării în ariile protejate din Republica Moldova [Guidelines for conservation management in protected areas in the Republic of Moldova]*. Chisinau, 2013.
4. Batir L, Chiseliţa O, Slanina V. Conservarea şi menţinerea bacteriilor cu proprietăţi importante pentru agricultura durabilă [Preservation and maintenance of bacteria with important properties for sustainable agriculture]. In: Corcimaru S., eds. *Potenţialul microbiologic pentru agricultura durabilă* [*Microbiological potential for sustainable agriculture*]. Chisinau, Ed:Ştiinţa, 2016, p.143-157.
5. Batir L, Slanina V. Microorganisms with a high antifungal activity after conservation. *Simpozionul ştiinţific naţional cu participare internaţională "Biotehnologii avansate – realizări şi perspective" [The national scientific symposium with international participation "Advanced biotechnologies - achievements and perspectives"]*. ed. IV-a. Chisinau, 3-4.10.2016. Available from: https://ibn.idsi.md/sites/default/files/imag_file/020-020.pdf [Accessed 01 May 2015].
6. Batir L, Slanina V. Antifungal activity of some strains of microorganisms after 3 and 6 years of lyophilization. *The International Conference „Life Sciences in the dialogue of generations: connections between universities, academia and business community"*. Chisinau. Available from: https://ibn.idsi.md/sites/default/files/imag_file/43_6.pdf [Accessed 01 May 2015].
7. Cupletscaia M, Netrusov A. Jiznesposobnost´ liofilizirovannykh mikroorganizmov posle 50 let khraneniia [Viability of lyophilized microorganisms after 50 years of storage]. *Microbiologiia*. Moscva; 2011. Available from: https://naukarus.com/zhiznesposobnost-liofilizirovannyh-mikroorganizmov-posle-50-let-hraneniya [Accessed 09 May 2015].
8. Sidorciuc A, Crasnova A. Sokhrannost´ cul´tur bakterii razlichnykh grup pri dlitel´nom khranenii v liofilizirovannom sostoianii [Preservation of cultures of bacteria of various groups during long-term storage in a lyophilized state]. *Rossiiscii veterinarnyi zhurnal*. 2016;3:22-25.
9. Slanina V, Balan (Batîr) L. Izolarea şi evaluarea diversităţii bacteriilor din apa lacurilor parcului „La izvor" [Isolation and evaluation of the diversity of bacteria in the water of the lakes of the "La izvor" park]. *Simpozion ştiinţific naţional cu participare internaţională: Biotehnologii moderne - soluţii pentru provocările lumii contemporane*. Chişinau, 20-21 mai, 2021:93. doi:10.52757/imb21.059
10. Salcher Michaela M, Šimek K. Isolation and cultivation of planktonic freshwater microbes is essential for a comprehensive understanding of their ecology. *Aquatic microbial ecology*. 2016;77:183-96. doi:10.3354/ame01796
11. Zarnea GR, Mihailescu S. *Principii şi tehnici de microbiologie generală [Principles and techniques of general microbiology]*. Bucureşti: 1992.
12. Gerkhard F. *Khranenie, subkul´tivirovanie, proverka chistykh kul´tur [Storage, subcultivation, verification of pure cultures]*. Moscva: Mir; 1983.
13. Kligler Iron Agar (7140), Product Information. Available from: http://biotrading.com/assets/productinformatie/acumedia/tds/7140.pdf [Accessed 02 May 2015].
14. Kligler's Iron Agar Test Principle, Procedure, Result. Available from: https://microbiologynote.com/kliglers-iron-agar-test-principle-procedure-result/ [Accessed 02 May 2015].

15. KIA Test - Principle, Media, Procedure, Results, Uses. Available from: https://microbenotes.com/kligler-iron-agar-test/ [Accessed 02 May 2015].
16. Triple Sugar Iron Agar TSI Agar. Available from: https://legacy.bd.com/europe/regulatory/Assets/IFU/Difco_BBL/226540.pdf [Accessed 02 May 2015].
17. Bogdan-Golubi N, Slanina V, Balan L, Ruginescu R. Molecular techniques for determining bacterial diversity in lake ecosystem. *The Scientific International Symposium "Advanced Biotechnologies Achievements and Prospects",* VIth edition. Chisinau, 3-4 October 2022:132-35. Available from: https://ibn.idsi.md/sites/default/files/imag_file/IGFPP_Symp._Biotehnologii_2022.pdf [Accessed 02 May 2015].
18. Contsevaia I. *Microbiologiia: cul´tivirovanie i rost bacterii [Microbiology: cultivation and growth of bacteria]*. Moscva: Desna Poligraf; 2017. Available from: https://old.gsu.by/biglib/GSU/%D0%91%D0%B8%D0%BE%D0%BB%D0%BE%D0%B3%D0%B8%D1%87%D0%B5%D1%81%D0%BA%D0%B8%D0%B9/10_Posobie2_zam81_44str_15ekz_%D0%9A%D0%BE%D0%BD%D1%86%D0%B5%D0%B2%D0%B0%D1%8F.pdf [Accessed 02 May 2015].
19. Batîr L, Slanina V, Sîrbu T, inventors; *Procedeu de conservare a tulpinii Bacillus cereus var. fluorescens cu activitate antifungică [Preservation process of the strain Bacillus cereus var. fluorescens with antifungal activity]*. Brevet de invenţie / Patent of invention MD 1071. April 30, 2017.
20. Uzunova-Doneva T, Donev T. Anabiosis and conservation of microorganisms. *Journal of culture collections*. 2005;4:17-28. Available from: https://nbimcc.org/JCC/2005/JCC0542/JCC0542S.htm [Accessed 07 May 2015].
21. Kapali S, Gade R, Shitole A, Aswathi S. Isolation and Characterization of *Pseudomonas fluorescens* and *Bacillus subtilis* and their *in vitro* Evaluation. *Advances in Life Sciences*. 2016;5(16):5856-59. doi:10.3390/agronomy10091428
22. Mulamattathil S, Bezuidenhout C, Mbewe M, Ateba C. Isolation of Environmental Bacteria from Surface and Drinking Water in Mafikeng, South Africa, and Characterization Using Their Antibiotic Resistance Profiles. *Journal of Pathogens*. 2014. doi:10.1155/2014/371208
23. Grabova A. Skrining shtammov bakterii roda *Bacillus* – aktivnykh antagonistov fitopatogenov bakterial'noy i gribnoi prirody [Screening of strains of bacteria of the genus *Bacillus* - active antagonists of bacterial and fungal phytopathogens]. *Mikrobiologichnyi zhurnal*. 2015;77(6):47-54. Available from: https://www.researchgate.net/profile/Anna-Grabova/publication/298046459_BACILLUS_STRAINS'S_SCREENING--ACTIVE_ANTAGONISTS_OF_BACTERIAL_AND_FUNGAL_PHYTOPATHOGENS/links/602b191892851c4ed575149a/BACILLUS-STRAINSS-SCREENING--ACTIVE-ANTAGONISTS-OF-BACTERIAL-AND-FUNGAL-PHYTOPATHOGENS.pdf [Accessed 08 May 2015].
24. Huda Z. Majeed. Antimicrobial activity of *Micrococcus luteus* Cartenoid pigment. *Journal of Science*. 2017;28(1). doi:10.23851/mjs.v28i1.xxx
25. Stadnichenko M. Perspektivy biologicheskogo kontrolia vozbuditelia botritioza na paslenovykh kul'turakh [Prospects for the biological control of the causative agent of botrythiosis on nightshade crops]. *Vestnik BGU.* 2011;(2):49-55. Available from: https://elib.bsu.by/handle/123456789/13855 [Accessed 08 May 2015].
26. Devi S, Kiesewalter H, Kovács R, Frisvad J, Weber T, et al. Depiction of secondary metabolites and antifungal activity of *Bacillus velezensis* DTU001. *Journal Synthetic and Systems Biotechnology*. 2019; 4(3):142-49. doi:10.1016/j.synbio.2019.08.002
27. Fan B, Blom J, Klenk H-P, Borriss R. *Bacillus amyloliquefaciens, Bacillus velezensis*, and *Bacillus siamensis* Form an "Operational Group *B. amyloliquefaciens*" within the B. subtilis Species Complex. *Front. Microbiol*. 2017;8:22.
28. Fan B, Wang C, Ding X, Zhu B, Song X, Borriss R. *AmyloWiki: an integrated database for Bacillus velezensis FZB42, the model strain for plant growth-promoting Bacilli*. Oxford: Database; 2019. Accessed January 1, 2019. baz071. doi:10.1093/database/baz071
29. Li-Ting W, Fwu-Ling L, Chun-Ju T, Hsiao-Ping K. *Bacillus velezensis* is a later heterotypic synonym of *Bacillus amyloliquefaciens*. *International Journal of Systematic and Evolutionary Microbiology*. 2008;58:671-75. doi:10.1099/ijs.0.65191-0

Date of receipt of the manuscript: 02/05/2023
Date of acceptance for publication: 28/09/2023

OH&RM ONE HEALTH & RISK MANAGEMENT

VOL. 4, ISSUE 4
2023

BIOLOGICAL SCIENCES

OPEN ACCESS

AN ANALYSIS OF COVID-19 INFECTIONS, RECOVERIES AND DEATHS IN RIVERS STATE, NIGERIA

Oludare ADEDIRE, Walter Chinaka JOHN, Afolabi Ola OLADEJO, Godfrey ONUWA, Jacob Jepthah MBAH

Federal College of Forestry Jos, Plateau State, Nigeria

Corresponding author: Oludare Adedire, e-mail: dharenss@gmail.com

DOI: 10.38045/ohrm.2023.4.04 CZU: 616.98:578.834.1(669)

Keywords: *disease, corona virus, COVID-19, rivers, Nigeria.*

Introduction. *The objective of the current study is to determine statistical difference in means of COVID-19 infected population, discharged cases and deaths resulting from COVID-19 pandemic and to estimate number of people that can survive it in Rivers State, Nigeria.* ***Material and methods.*** *In this study, extraction of infected population, discharged cases and deaths from COVID-19 was carried out from data obtained from Nigeria Centre for Disease Control which covered a period of 572 days. The test for significance of means among the variables was done using one-way Analysis of Variance followed by Tukey HSD test. The two variables whose means do not significantly differ were tested for strength of relationship and dependence using correlation analysis and cubic polynomial function respectively.* ***Results.*** *Results showed that there is statistically significant difference between infected population, discharged cases and deaths from COVID-19 as obtained from one-way ANOVA (F(2, 1710)=121.958, p=0.000). Use of Tukey HSD test indicated that means of infected population and discharged cases do not differ from each other (p>0.01); however, mean deaths from COVID-19 significantly differ from others at 99% confidence intervals (p<0.01). There was weak positive relationship between deaths due to COVID-19 and other two groups (r=0.360, n=571, p=0.000). However, moderate positive correlation existed between COVID-19 infected population and discharged population (r=0.566, n=571, p=0.000) at 99% confidence interval.* ***Conclusions.*** *Mean of deaths from the study population was significantly different from means from both infected population and discharged cases. This implies that only few people died from COVID-19 in Rivers State Nigeria when compared to those who got infected and recovered from COVID-19 pandemic.*

Cuvinte-cheie: *boală, coronavirus, deces, COVID-19, râuri, Nigeria.*

ANALIZA INFECȚIILOR, RECUPERĂRILOR ŞI A DECESELOR CAUZARE DE COVID-19 ÎN LUNCA R URILOR DIN NIGERIA

Introducere. *Obiectivul studiului este de a determina diferența statistică între mediile populației infectate cu COVID-19, cazurile externate și decesele rezultate în urma pandemiei și de a estima numărul de persoane care pot supraviețui acesteia în lunca râurilor, din Nigeria.* ***Material și metode.*** *În acest studiu extragerea populației infectate, a cazurilor externate și a deceselor din cauza COVID-19 a fost efectuată în baza datelor obținute de la Centrul Nigeria pentru Controlul Bolilor, care a acoperit o perioadă de 572 de zile. Testul pentru semnificația mediilor dintre variabile a fost realizat folosind analiza variației unidirecționale urmată de testul Tukey HSD. Cele două variabile ale căror medii nu diferă semnificativ au fost testate pentru puterea relației și dependența folosind analiza corelației și, respectiv, funcția polinomială cubică.* ***Rezultate.*** *Rezultatele au arătat că există o diferență semnificativă statistic dintre populația infectată, cazurile externate și decesele cauzate de COVID-19, obținute prin testul ANOVA unidirecțional (F(2, 1710)=121,958, p=0,000). Utilizarea testului Tukey HSD a indicat că mediile populației infectate și cazurile externate nu diferă unele de altele (p>0,01). Cu toate acestea, decesele medii cauzate de COVID-19 diferă semnificativ de altele la intervale de încredere de 99% (p<0,01). A existat o relație pozitivă redusă între decesele cauzate de COVID-19 și alte două grupuri (r=0,360, n=571, p=0,000). Prin urmare, a existat o corelație pozitivă moderată între populația infectată cu COVID-19 și populația externată (r=0,566, n=571, p=0,000) la un interval de încredere de 99%.* ***Concluzii.*** *Media deceselor din populația de studiu a fost semnificativ diferită de media atât de la populația infectată, cât și de la cazurile externate. Aceasta implică faptul că doar puțini oameni au murit din cauza COVID-19 în lunca râurilor din Nigeria, în comparație cu cei care s-au infectat și s-au recuperat după pandemia de COVID-19.*

INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a virus strain responsible for Corona virus disease 2019(COVID-19). Its first case was discovered in year 2019 in Wuhan, Hubei province of China and has spread to different parts of the world. Since Nigeria's first index case arrived Lagos State on the 27th February 2020, other states of Federal Republic of Nigeria have recorded daily epidemiological case of COVID-19. Rivers State being one of the thirty-six states in Nigeria had its first laboratory confirmed case announced by Nigeria Centre for Disease Control on the 25th March 2020. As part of the proactive measures taken to curtail the spread of the virus, Rivers State government announced closure of all schools within the state and restricted activities related to religious groups, cinemas, night clubs, public parks, weddings and burials (1, 2). Further measures to curb the spread of the virus included indefinite closure of the sea, air and land borders on the 26th March 2020 (3) and on the 28th March 2020, the state government announced indefinite closure of all markets (4).

Some studies have been conducted on COVID-19 for some states in Nigeria (5). Alasia and Maduka (6) investigated prevalence and pattern of COVID-19 infection in Rivers State with emphasis on healthcare workers and concluded that prevalence of COVID-19 among healthcare workers was high. Robinson (7) also assessed COVID-19 safety precautions in radio-diagnostic centres in capital city of Rivers State and concluded that personal protective equipment (PPE) were generally unavailable in many radio-diagnostic centres thereby posing significant increase in risks of COVID-19 infections. In findings of Uzosike et al. (8), they also highlighted experiences of health workers in Rivers State and factors that could improve their performance in handling COVID-19 patients. A positive perspective of the effect of COVID-19 lockdown on farmers in Rivers State, Nigeria was also examined by Anagah (9) where he discussed how the sales of agricultural produce led to very high profits due to surge in demands relative to COVID-19 lockdown.

Investigation of clinical and epidemiological characteristics of some hospitalized SARS-CoV-2 positive patients in Rivers State (10) indicates that patients of COVID-19 disease have leading symptoms of fever, fatigue, dry cough, dyspnea, diarrhoea and vomiting with hypertension, diabetes and increasing age associated severe disease and death in the study population and therefore concluded that such epidemiological and clinical observations of COVID-19 patients in Nigeria is similar to the patterns in Africa and across the globe.

On daily and weekly bases, results of laboratory confirmed COVID-19 cases were published by Nigeria Centre for Disease Control (NCDC) for Rivers State and such painstaking efforts require laboratory tests with use of chemicals and other resources. *The aim of this study* is to statistically determine level of association between infected population, discharged cases and deaths from COVID-19 in Rivers State, Nigeria. Specific objective of this study is to estimate the number of people that can survive the disease whenever infected population is known. This can help gain insight into pattern of spread, recovery and deaths from COVID-19 in the study area.

MATERIAL AND METHODS

Study Area

The study areas Rivers State with estimated population of 7,303,924 as at the year 2016 and is located in south-south geopolitical zone of Nigeria (11). It consists of twenty-three local government areas and has its borders with Atlantic Ocean, Akwa Ibom, Imo, Abia, Bayelsa, Delta and Anambra states. Rivers State has average temperatures ranging between 25°C and 28°C, relative humidity usually above 60% throughout the year and rainfall is seasonal occurring between the months of March and November with peak of wet season usually in July. The inland part of the state around the coastal areas consists of tropical rainforest with typical mangrove swamp environments and the capital city of Rivers State is Port Harcourt (12) which is regarded as the commercial hub of Nigerian oil industry.

Rivers State was created by Nigerian government in the year 1967 and it comprises of minority ethnic groups, some of whom are Ikwerre, Ogoni, Ijaw and Okrika people (12). Presence of petroleum industry has increased revenue for Nigerian government and has attracted other ethnic groups and nationalities.

Epidemic Data

The data for this study was obtained from Nigeria Centre for Disease Control (NCDC). It consists

of daily records of COVID-19 disease data from the first day of disease detection in Rivers State which is 25th March 2020 to 17th October 2021. The period covered by the data for this study includes both the period of non pharmaceutical approach and the period of vaccination of some class of susceptible population in Rivers State. However, due to insufficient number of vaccines at the time of this study, quite a large number of susceptible individuals have not been vaccinated. This still has significant drawbacks on the efforts required to curtail the spread of the virus.

Data Analysis

This research investigated test for significance of means among variables using one-way Analysis of Variance (ANOVA) and Tukey HSD test. Correlation analysis and cubic polynomial function were used to test for strength of relationship and dependence respectively among variables. Data analysis and results were obtained with the aid of IBM Statistical Package for Social Sciences (version 23). Research hypothesis for this study consists of null hypothesis which states that there are no statistically significant differences between means of infected population, discharged cases and deaths due to COVID-19. The alternate hypothesis of the study states that there are statistically significant differences between means of infected population, discharged cases and deaths due to COVID-19.

Model Specification

Let X_{ij}be dependent variable which represents each infected COVID-19 patient in each treatment group$j$representing infected population (lab confirmed), discharged cases or deaths due to COVID-19 and μ represent the population mean for treatment effect β_j in the group j, then mathematical model representing one-way ANOVA for a completely randomised design is given by $X_{ij} = \mu + \beta_j + \xi_{i\,(j)}$where $\xi_{i\,(j)}$ is a random error or effect associated with other extraneous variables on COVID-19 infected patients in treatment group j representing infected population (lab confirmed), discharged cases or deaths due to COVID-19. It is assumed that $\xi_{i\,(j)}$is independent and normally distributed with mean zero and equal variance σ^2 written in short notation as $\xi_{i\,(j)} \sim N\,(0, \sigma^2)$.

Pearson Product Moment Correlation (PPMC) of the degree of relationship between variable V representing infected population (lab confirmed) and variable D representing death due to COVID-19 in Rivers State, Nigeria is defined by

$$r = \frac{N\sum VD \; - \; (\sum V)\,(\sum D)}{\sqrt{[N\sum V^2 \; - \; (\sum V)^2]} \;\; . \;\; \sqrt{[N\sum D^2 \; - \; (\sum D)^2]}}$$

where r is the correlation coefficient such that $-1 \leq r \leq 1$.

Define function $f(v)$ simply denoted by F as a dependent variable on independent variable v, then cubic polynomial function is of the form

$$F = p + qv \; + rv^2 + \; s\,v^3$$

which can be referred to as polynomial function of degree 3 where **p**, **q**, **r** and **s** are constants of the functional representation **F** for independent variable **v**.

RESULTS

Graphical analyses of results showing data for daily infected population (lab confirmed), daily discharged cases and daily deaths due to COVID-19 in Rivers State are displayed in Figures 1, 2 and 3.

The contents of table 5 indicate that relationship showing dependence of discharged cases on infected population could be established using polynomial function of degree 3. Simple explanation of this is that data set representing COVID-19 infected population and discharged cases from Rivers State, Nigeria could be fitted to give fairly good approximation of discharged patients whenever daily infected cases are known. From results shown in Table 5, relationship between the COVID-19 infected population and discharged case can be represented by the cubic polynomial given by $F = 4.673 + 1.148\,V - \; 0.007V^2 + \; 1.7265 \; \times 10^{-5}V^3$ where F represents group of people which were discharged free from COVID-19 and V represents variable for COVID-19 infected population in Rivers State, Nigeria.

DISCUSSIONS

It can be observed that patterns shown in Figure 1 follow similar trend as observed in Figure 2 but with some little differences when juxtaposed, but Figure 3 showed very low number of people when compared with Figures 1 and 2. This implies that deaths due to COVID-19 did not have similar pattern with both infected cases and discharged population. It can be observed (fig. 4) that average of 22 people were confirmed infected with COVID-19 daily in Rivers State, Nigeria. While av-

erage of 21 patients was discharged, deaths due to COVID-19 have an average of 1 death per day.

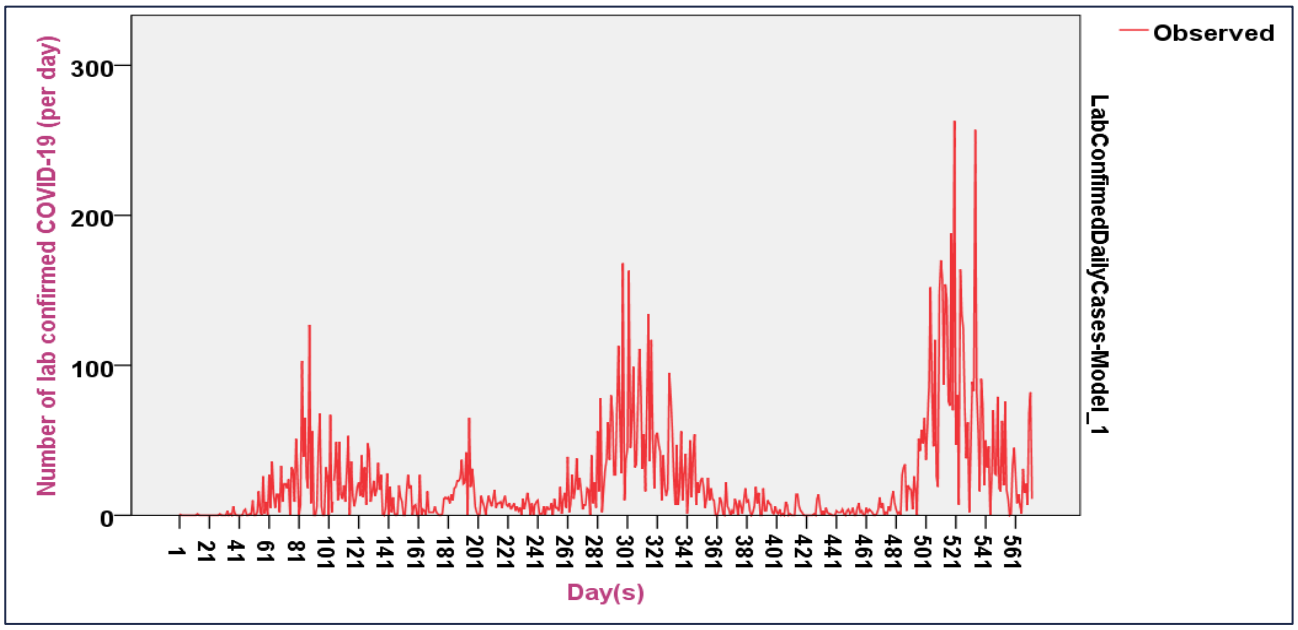


Figure 1. Daily laboratory confirmed COVID-19 cases in Rivers State (25th March 2020 - 17th Oct 2021).

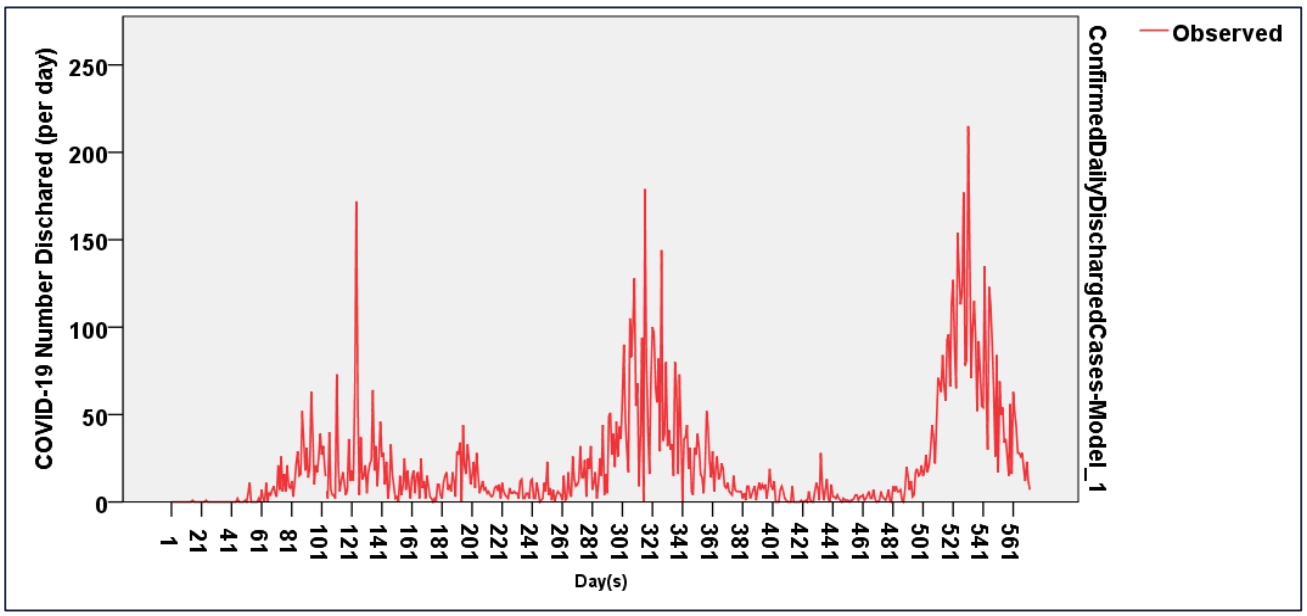


Figure 2. Daily discharged cases (formerly infected with COVID-19) in Rivers State (25th March 2020 - 17th Oct 2021).

In order to determine whether there are statistically significant differences between means of infected population (lab confirmed), discharged cases and deaths due to COVID-19, results for One-Way Analysis of Variance are shown in Table 1. Furthermore, from results displayed in Table 1, it can be seen that there is a statistically significant difference between COVID-19 infected population, discharged cases and deaths from COVID-19 ($F(2,1710)=121.958$, $p=0.000$). However, results in Table 1 do not indicate specific groups which were significantly different from others. Since it has been affirmed from the results presented in Table 1 that at least two groups were different, it is pertinent to show which of the groups differ from others with aid of post hoc test. Thus, using Tukey post hoc test, results are shown in Table 2 which are multiple comparisons.

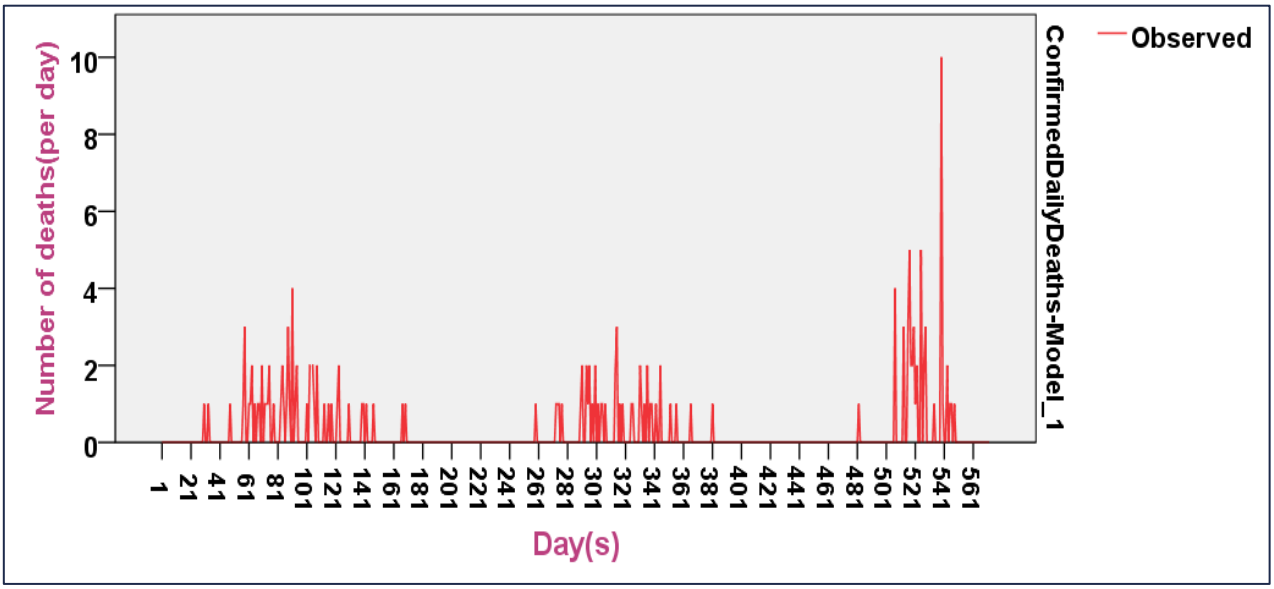


Figure 3. Deaths per day due to COVID-19 in Rivers State (25th March 2020 - 17th Oct 2021).

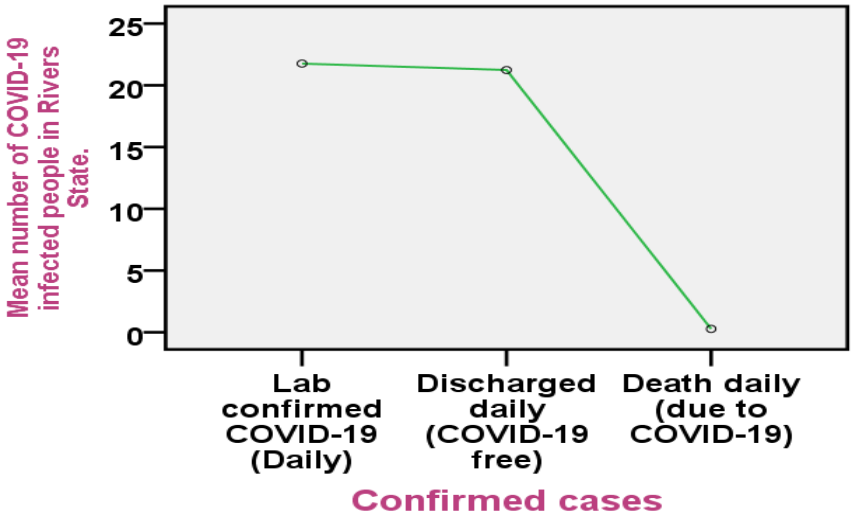


Figure 4. Mean number of COVID-19 infected, discharged and deceased cases in Rivers State (25th March 2020 - 17th Oct 2021).

Table 1. One-way ANOVA for COVID-19 infected, discharged and deceased cases in Rivers State (25th March 2020 - 17th Oct 2021).

Model	Sum of squares	df	Mean square	F	p-value
Between Groups	171554.891	2	85777.445	121.958	0.000
Within Groups	1202705.110	1710	703.336		
Total	1374260.001	1712			

It can be observed that there is no statistically significant difference between the group representing COVID-19 infected population (lab confirmed) and the group representing discharged cases (p=0.941) but there is statistically significant difference between COVID-19 infected population (lab confirmed) and group representing deaths due to COVID-19 (p=0.000).

Also, there was statistically significant difference between the groups representing discharged cases and daily deaths due to COVID-19 in Rivers

Table 2. Tukey post hoc test of multiple comparison.

(i) COVID-19 infected population (lab confirmed)	(j) COVID-19 infected population (lab confirmed)	Mean Difference(i-j)	Std. Error	p-value	99% confidence Interval	
					LBound	UBound
COVID-19 infected population (lab confirmed)	Discharged case (COVID-19 free)	0.522	1.570	0.941	-3.16	4.20
	Deathdaily (due to COVID-19)	21.485	1.570	0.000	17.80	25.17
Discharged case (COVID-19 free)	COVID-19 infected population (lab confirmed)	-0.522	1.570	0.941	-4.20	3.16
	Deathdaily (due to COVID-19)	20.963	1.570	0.000	17.28	24.65
Deathdaily (due to COVID-19)	Discharged case (COVID-19 free)	-21.485	1.570	0. 000	-25.17	-17.80
	COVID-19 infected population (lab confirmed)	-20.963	1.570	0.000	-24.65	-17.28

Table 3. Strength of relationship between infected population and deaths due to COVID-19.

		Infected population (lab confirmed)	Deaths due to COVID-19	p-value
Infected population (lab confirmed)	Pearson Correlation	1	0.360+	0.000++
Deaths due to COVID-19		0.360+	1	0.000++

\+ Value correlation coefficient r
++ Significant at α = 0.01 level

Table 4. Strength of association between infected population and discharged cases.

		Infected population (lab confirmed)	Dailydischarged cases	p-value
Infected population (lab confirmed)	Pearson correlation	1	0.566+	0.000++
Dailydischarged cases		0.566+	1	0.000++

\+ Value correlation coefficient r
++ Significant at α = 0.01 level

Table 5. Cubic polynomial approximation of daily discharged case from COVID-19 infected population.

Model	B	Std Error	Beta	t	p-value
(constant)	4.673	1.519		3.077	0.002
Daily infected case	1.148	0.119	1.265	0.9643	0.000
Daily infected case (square)	-0.007	0.002	-1.343	-4.485	0.000
Daily infected case (cube)	1.7265×10^{-5}	0.000	0.672	3.316	0.010

State (p=0.000). This implies that deaths due to COVID-19 significantly differ from both COVID-19 infected population and discharged cases in Rivers State, Nigeria (p=0.000). It is worthwhile to determine degree of relationship between the categories of COVID-19 infected population, discharged cases and deaths due to COVID-19.

In order to determine the degree of relationship between the categories of COVID-19 infected population, discharged cases and deaths due to COVID-19, results for correlation analysis between COVID-19 infected population and deaths due to COVID-19 are shown in Table 3. Though relationship exists between COVID-19 infected population and deaths due to C0VID-19, it is a weak positive relationship (r=0.360, n=571, p=0.000) at 99% confidence interval.

From results in Table 4, the degree of relationship indicated a moderately strong relationship between the two groups. This implies that the relationship between COVID-19 infected population and daily discharged cases exhibited moderately positive relationship (r=0.566, n=571, p=0.000) at 99% confidence interval.

From results obtained using One-Way ANOVA and Correlation Analysis, it may not be necessary to further analyse the association between deaths and other groups. However, more investigation could still be explored with respect to relation ship between characteristics of COVID-19 infected population and discharged cases; hence results from cubic polynomial functional relationship are displayed (tab. 5).

The essence of Table 5 is the formulation of mathematical equation which can be used to determine number of people F that may be discharged free from Covid-19 whenever population of COVID-19 infected people V are known in Rivers State Nigeria. For instance, if 800 people are COVID-19 infected, then set V=800 in the derived equation to obtain an approximate $F \approx 5283$ which implies that about 5283 people may be discharged free from COVID-19. This means that knowing lab confirmed cases, the formulated mathematical equation can facilitate estimation of the number of people that can survive the disease.

While the studies conducted by Alasia and Maduka centred on healthcare workers without considering discharged cases, this study concurs with their conclusion and extends to a wider population, suggesting a high spread of COVID-19 among the population. However, the succour provided by this study is the possibility of high recovery rate from COVID-19 in Rivers State. The reasons, however, could be due to the non-adherence of the infected population to measures aimed at curtailing the spread of the virus.

CONCLUSIONS

1. In this study, analysis of infections, recoveries and deaths from COVID-19 in Rivers State, Nigeria was carried out. The results from One-Way Analysis of Variance showed that there was a statistically significant difference between COVID-19 infected population (lab confirmed), discharged cases and deaths due to COVID-19 (F(2,1710)=121.958, p=0.000). Tukey post hoc test indicated that deaths from COVID-19 significantly differ from both infected population (lab confirmed) and discharged cases (p=0.000). However, there was no significant difference between the COVID-19 infected population (lab confirmed) and discharged population (p=0.941).
2. Results from Pearson Product Moment Correlation to determine the degree of relationship between deaths due to COVID-19 and the other two groups showed that there was a weak positive relationship (r=0.360, n=571, p=0.000) at 99% confidence interval. However, moderate positive correlation existed between COVID-19 infected population and discharged cases (r=0.566, n=571, p=0.000) at 99% confidence interval. Also, a cubic polynomial equation was derived in order to predict discharged cases whenever infected cases are known in Rivers State, Nigeria.

CONFLICTS OF INTEREST

Authors declare that they do not have conflicts of interest.

ETHICAL APPROVAL

The Nigeria Centre for Disease Control approved the study protocol and data in accordance with Nigerian legislation and the ethical standards using multi-sectoral emergency oper

ation centre (EOC) as contained in COVID-19 Outbreak in Nigeria Situation Report S/N 001; 29.02.2020.

REFERENCES

1. Nwakaudu S. Coronavirus: Rivers government closes schools. *Vanguard Newspaper*. Available from: https://www.vanguardngr.com/2020/03/coronavirus-rivers-government-closes-schools[Accessed21 March 2020].
2. Odiegwu M. BREAKING: Wike announces partial lockdown of Rivers over COVID-19. *The Nation Newspaper*. Available from: https://thenationonlineng.net/breaking-wike-announces-partial-lockdown-of-rivers-over-covid-19[Accessed23 March 2020].
3. Odiegwu M. COVID-19: Wike locks down Rivers, closes all borders. *The Nation Newspaper*. Available from:https://thenationonlineng.net/covid-19-wike-locks-down-rivers-closes-all-borders[Accessed25 March 2020].
4. Odiegwu M. 19-year-old Rivers index case traveled to Italy, Greece, France. *The Nation Newspaper*. Available from:https://thenationonlineng.net/19-year-old-rivers-index-case-traveled-to-italy-greece-france [Accessed26 March 2020].
5. Adedire O, Ndam JN. A model of dual latency compartments for the transmission dynamics of COVID-19 in Oyo State Nigeria. *J Engr Appl Sci Lett*. 2021;4(1):1-13.
6. Alasia DD, Maduka O. Prevalence and pattern of COVID-19 among healthcare workers in Rivers State Nigeria. *OccDisEnvir Med*. 2021; 9:20-32.
7. Robinson ED. Assessment of COVID-19 safety precautions in radio diagnostic centres in Port-Harcourt Rivers State. *The Nig Health Jour*. 2020;20(4):131-42.
8. Uzozike TC, Dan-Jumbo A, Bob-Manuel M, Alali AA, Lawson DS. Care of the COVID-19 Patients: Experiences of health workers in Rivers State Nigeria. *Int J Trop Dis & Health*. 2020;41(23):1-15.
9. Anagah FI. Effect of COVID-19 Lockdown on Farmers in Rivers State, Nigeria: Positive Perspective. *Asian J Agricl Ext Econs& Soc*. 2020;38(5):56-59.
10. AlasiaD, Owhonda G, MadukaO, NwadiutoI,AruguG, Tobin-WestC, et al. Clinical and epidemiological characteristics of 646 hospitalised SARS-Cov-2 positive patients in Rivers State Nigeria: a prospective observational study. *Pan Afri Med J.*2021;38(25). doi:10.11604/pamj.2021.38.25.26755
11. National Bureau of Statistics (2017): Demographic statistics bulletin, Federal Republic of Nigeria. Vol 3. Available from:www.nigerianstat.gov.ng [Accessed 01 September 2020].
12. Kari EE. The languages of Rivers State of Nigeria: An Overview. *Marang J Lang Lit*. 2019; 31:1-22.

Date of receipt of the manuscript: 21/05/2023
Date of acceptance for publication: 15/09/2023

OH&RM ONE HEALTH & RISK MANAGEMENT

VOL. 4, ISSUE 4
2023

MEDICAL SCIENCES

OPEN ACCESS

BIBLIOMETRIC ANALYSIS OF LITERATURE RELATING TO NOISE POLLUTION REPORTED OVER THE PERIOD 2001-2020

Erhan KAYA[1], Musa ŞAHIN[2], Onur ACAR[3]

[1]Kahramanmaras Sutcu Imam University, Faculty of Medicine, Kahramanmaras, Turkey
[2]Public Health Directorate of Adana, Turkey
[3]Public Health Directorate of Ağrı, Turkey

Corresponding author: Erhan Kaya, e-mail: erhan.ky3@outlook.com

DOI: 10.38045/ohrm.2023.4.05 CZU: 613.164+613.644

Keywords: *noise pollution, noise, traffic noise.*

Introduction. *In the new globalized world, noise pollution has started to become a public health problem. Health issues from noise pollution include hearing problems, cardiovascular disorders, and sleep disturbances.*

Material and methods. *We searched for publications about noise pollution in the Web of Science database. A total of 2722 papers were identified, published between 2001 and 2020, 1815 of them were analyzed. VOSviewer (version 1.6.11) tool was used for bibliometric web visualizations.*

Results. *When a trend analysis was applied to the articles by year, a statistically significant increase was detected. The United States contributed to the most publications (15.3%). Scotland (6.62), Singapore (4.26), and Ireland (4.02), to the most frequent publications per million of inhabitants. Most articles on noise pollution were published in the journal Applied Acoustics (3.2%). The three keywords we used were "noise pollution", "noise" and "traffic noise".*

Conclusions. *This study showed that there has been a trend of an increasing number of articles on noise pollution in the last 20 years, also it can be considered that this bibliometric study will help researchers as it provides summary for current research.*

Cuvinte-cheie: *poluare fonică, zgomot, zgomot din trafic.*

ANALIZA BIBLIOMETRICĂ A LITERATURII PRIVIND POLUAREA FONICĂ, RAPORTATĂ ÎN PERIOADA 2001–2020

Introducere. *În noul context al unei lumi globalizate, poluarea fonică tinde să devină o ameninţare pentru sănătatea publică. Problemele de sănătate, cauzate de poluarea fonică, includ probleme de auz, dereglări cardiovasculare şi tulburări de somn.*

Material şi metode. *Am căutat publicaţii despre poluarea fonică în baza de date Web of Science. Au fost identificate în total 2722 de lucrări, publicate între 2001 şi 2020, dintre care au fost analizate 1815. Instrumentul VOSviewer (versiunea 1.6.11) a fost folosit pentru vizualizările web bibliometrice.*

Rezultate. *Analizându-se tendinţele articolelor apărute anual, s-a constatat o creştere semnificativă statistic. Statele Unite au contribuit cu cele mai multe publicaţii (15,3%). Scoţia (6,62), Singapore (4,26) şi Irlanda (4,02), cu cele mai frecvente publicaţii la un milion de locuitori. Cele mai multe articole despre poluarea fonică au fost publicate în revista Applied Acoustics (3,2%). Cele trei cuvinte-cheie, pe care le-am folosit, au fost „poluare sonoră", „zgomot" şi „zgomot din trafic".*

Concluzii. *Studiul a demonstrat că există o tendinţă de creştere a numărului de articole despre poluarea fonică în ultimii 20 de ani. Putem considera acest studiu bibliometric un suport eficient pentru cercetătorii din domeniu, întrucât pune la dispoziţie un rezumat care facilitează cercetările curente.*

INTRODUCTION

Noise is defined as a disturbing and irritating mixture of loud sounds which can cause temporary or permanent damage to humans and animals (1). The World Health Organization (WHO) has declared noise pollution to be the third most dangerous type of environmental pollutant after air and water pollution (2).

All countries, especially developing countries, are affected by many pollutants in the environment as a result of urbanization and industrialization (3). Noise pollution has increased in intensity with the rise in the industry since the industrial revolution, and with the rise of human activities a rapid urbanization increased with a consequent significant deterioration of exposed population health status. This rapid increase in industrialization, urbanization, and transportation systems has led to currently high levels of noise pollution. Most epidemiological studies have found that road traffic and communities are specifically the main sources of the noise pollution (4-7).

The recent literature focused on noise is important because it demonstrates that annoyance and disturbance due to noise are associated with a high incidence of diseases through harm to human health (8). Noise can cause auditory and non-auditory health effects, both psychological and physiological, and evidence for non-auditory effects was increasing in more recent years (4). According to WHO (2012) report, noise pollution can cause health problems such as cardiovascular diseases, cognitive impairment, sleep disturbance, tinnitus, and discomfort. Increased noise sensitivity can lead to psychiatric disorders such as anxiety and depression (9). Generally, human-generated noise also has a deleterious impact on natural life and has become an ecological pollutant as well (10).

Bibliometrics is the statistical analysis of scientific publications, about a specific topic or research field and disclosing the most effective publications, countries, authors, collaborations between institutions, and active journals (11, 12). Furthermore, it presents the summarized data and enables researchers to evaluate the current trends of the data (13). In addition to statistics, bibliometric indicators can provide insight into research implications, knowledge networks, and information distribution, which saves readers and writers time in terms of literature review by providing a summary of the literature (14).

The present study adopted a scientific analysis through the Web of Science (WoS) database to provide researchers and practitioners with an advanced review of noise pollutant-related studies. This method has the advantages of information retrieval as a whole to highlight the progression, hotspots, and boundaries of publications.

In this study, bibliometric analysis was conducted using newly developed visualization tools (e.g. VOSviewer) to map the global research status and vanguard trends of Public and Environmental Health research from multiple perspectives.

MATERIAL AND METHODS

The methodology of this study was planned with reference to similar studies in the literature (11, 13, 15).

Data sources

Bibliometric data were collected from the Web of Science (WoS) database platform. The literature review was performed using “noise pollution” as the keyword in the Title search section. The articles meeting the criteria were downloaded from the WoS database (access date: 19.08.2021) and analyzed using bibliometric methods.

The date range was set as January 2001 to December 2020. The document type included the articles and reviews. Proceeding paper, book chapter, editorial materials, meeting abstract, letter, and other document types were excluded from the study. VOSviewer was used to visualize the studies and form a network map of references, keywords, and citations. Bibliometric web visualizations were made using the VOSviewer (version 1.6.11). VOSviewer is a widely used software tool presenting visualization maps with connections by combining items such as countries, authors, journals, or keywords. The lines between the items and the thickness of the lines indicate the strength of the connection between them and clustering between items is also shown.

Countries that were the sources of at least 1% of the total number of articles in the date range included in our study were accepted as the main active countries (16). Analysis was performed by finding the total number of articles, the number of articles per million population, gross domestic product per capita ($) (accessed from

https://www.cia.gov/the-worldfactbook/countries/'website), total citations, average citations, and h index values for each country. The Science Citation Index Expanded (SCIE) and Emerging Sources Citation Index data of the analyzed articles were recorded.

Data analyses

The data obtained in the study were analyzed using SPSS vn.15 software (Statistical Package for Social Sciences, version 15, SPSS Inc., Chicago, IL, USA). Values were given as frequency and percentage. Regression analysis was performed to determine the trend of the number of published articles by years. The statistical significance level was accepted as 0.05.

RESULTS

Using the WoS database, a total of 2722 papers were identified published between January 2001 and December 2020. After the exclusion of non-original articles and non-review papers, 1905 papers remained, then in the second stage, papers not in English language or not directly relating to noise pollution were excluded, leaving a total of 1815 papers for our sample. The journal index distribution of the published articles was as follows: Science Citation Index Expanded (1415), Social Sciences Citation Index (119), Emerging Sources Citation Index (227), Book Citation Index–Science (37), Book Citation Index-Social Sciences and Humanities (11), and Arts and Humanities Citation Index (7).

When regression analysis was applied to the number of articles by year, a statistically significant increase was detected, from 16 in 2001 to 302 in 2020, showing an 18.9-fold increase ($p<0.001$). In the research, it was seen that there was an increasing trend in the number of articles over the years. The distribution of the number of articles by year is shown in Figure 1.

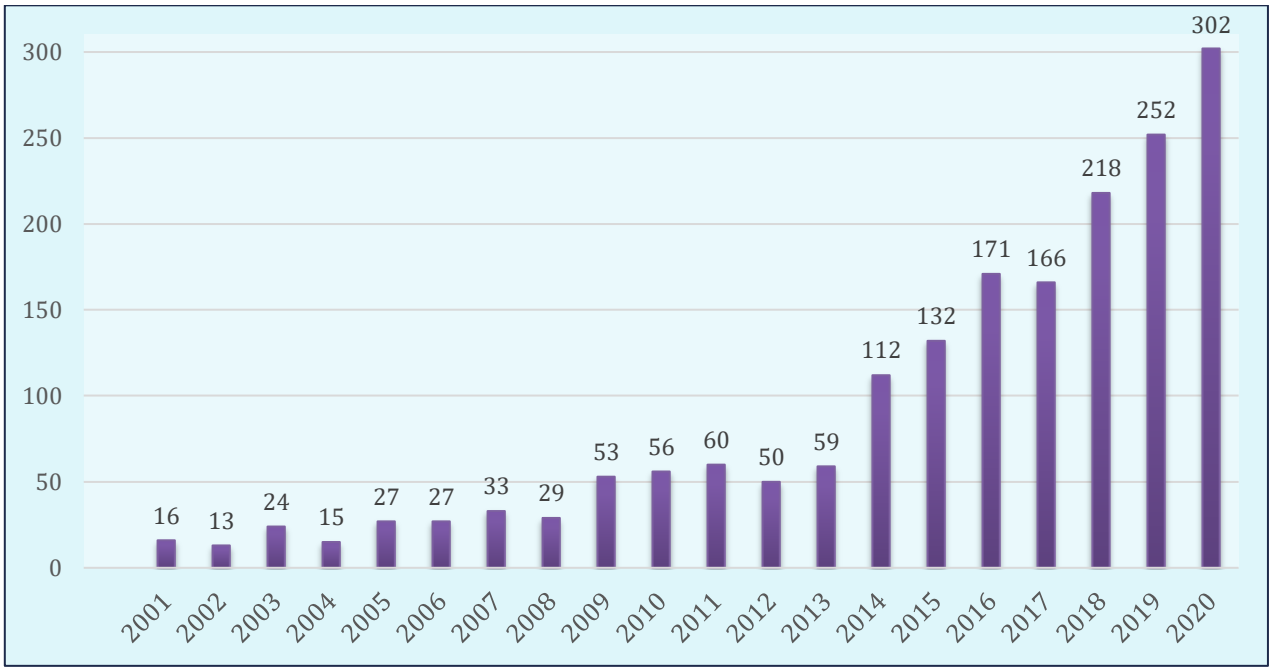


Figure 1. The distribution of papers by year and frequency, related to noise pollution.

The five most cited articles on noise pollution were listed. The authors of these articles were respectively, Stansfeld S.A. et al. (500 citations), Miedema H.M.E. et al. (458 citations), Francis Clinton D. et al. (329 citations), De Nazelle Audrey et al. (320 citations), and Rowe D. Bradley (308 citations) (tab. 1).

The co-authorship author's network map is shown in Figures 2-5.

The number of articles by country, number of articles per million population of productive countries, gross domestic product per capita ($) values of countries, total citations, average citation values, and adjusted h index values for each country are shown in Table 2.

Table 1. Top-10 most commonly-cited articles related to noise pollution.

Rank	Article Title	Authors	Institution	Journal	Year	C	CPY	JIFS
1	Noise pollution: non-auditory effects on health	Stansfeld S.A. et al.	Uni of London Queen Mary	*British Medical Bulletin*	2003	500	27.8	2.804
2	Annoyance from transportation noise: Relationships with exposure metrics DNL and DENL and their confidence intervals	Miedema H.M.E. et al.	Netherlands Organization Applied Science Research	*Environmental Health Perspectives*	2001	458	22.9	8.049
3	Noise pollution changes avian communities and species interactions	Francis Clinton D. et al.	Uni of Colorado	*Current Biology*	2009	329	27.4	9.193
4	Improving health through policies that promote active travel: A review of evidence to support integrated health impact assessment	De Nazelle Audrey et al.	Uni Pompeu Fabra	*Environment International*	2011	320	32	7.943
5	Green roofs as a means of pollution abatement	Rowe D. Bradley	Uni Michigan State	*Environmental Pollution*	2011	308	30.8	5.714
6	How and why environmental noise impacts animals: an integrative, mechanistic review	Kight Caitlin R. et al.	Uni of Exeter	*Ecology Letters*	2011	292	29.2	8.699
7	A synthesis of two decades of research documenting the effects of noise on wildlife	Shannon Graeme et al.	Uni Colorado State	*Biological Reviews*	2016	275	55	10.288
8	Potential public health hazards, exposures and health effects from unconventional natural gas development	Adgate John L. et al.	Uni Colorado Denver	*Environmental Science & Technology*	2014	272	38.8	7.149
9	Triboelectrification-based organic film nanogenerator for acoustic energy harvesting and self-powered active acoustic sensing	Yang Jin et al.	Georgia Institute of Technology	*Acs Nano*	2014	256	36.6	13.903
10	Environmental impact of wind energy	Saidur R. et al.	Uni Malaya	*Renewable & Sustainable Energy Reviews*	2011	251	25.1	10.556

JIFS: Journal impact factor score 2018; C: citations; CPY: citations per year

A significant percentage of the articles were from the USA (15.317%) and China (14.490%). The countries with the most articles per million population were determined to be Scotland (6.63), Singapore (4.26), and Ireland (4.02), respectively. In terms of the average number of citations, the top three countries were the Netherlands (44.96), Switzerland (39.90), and England (31.09).

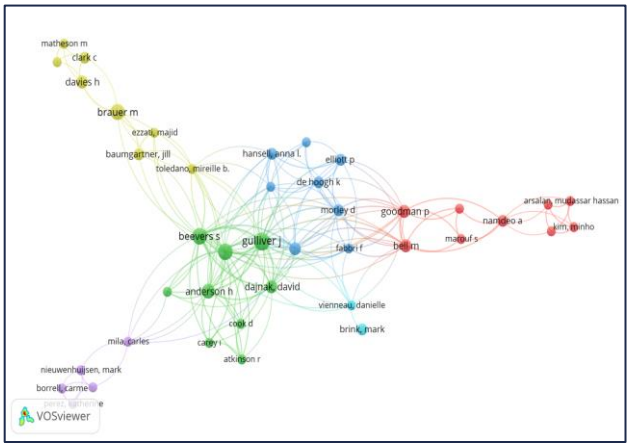


Figure 2. Co-authorship authors network visualization map.

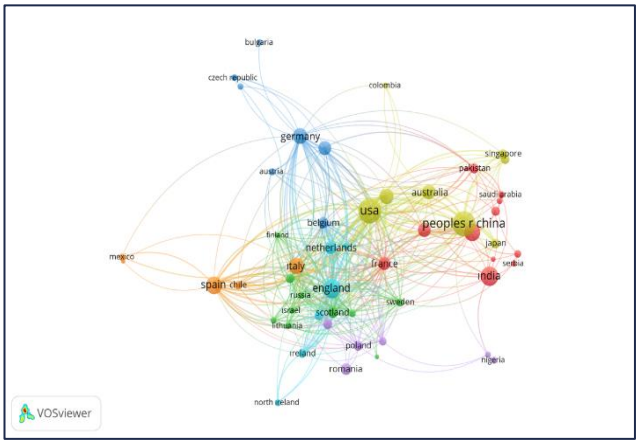


Figure 3. Co-authorship country network visualization map.

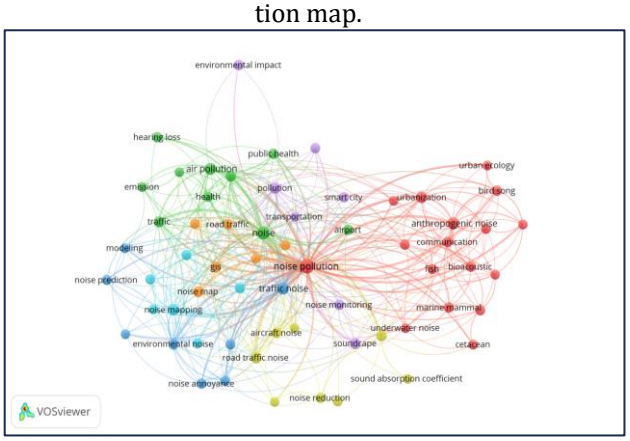


Figure 4. Author keywords co-occurrence network visualization map.

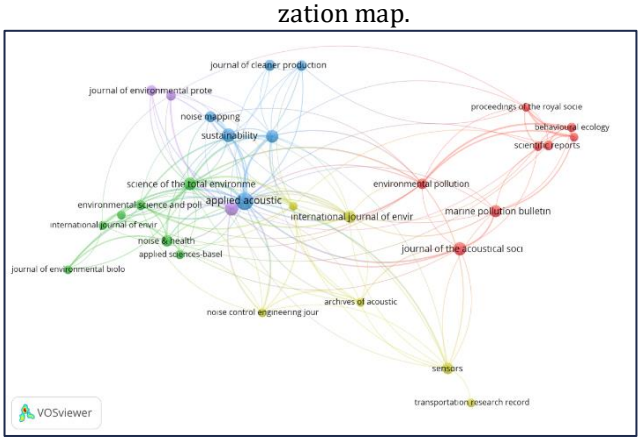


Figure 5. Bibliographic coupling of journals network visualization map.

For countries with a minimum of five papers, a country co-authorship visualization map was created in the VOSviewer application. From a total of 109 countries, 55 countries produced more than five publications. The visualization map of these countries with a minimum number of five papers can be seen in Figure 3.

According to Table 3, the three most used keywords used in the articles were noise pollution (25.179%), noise (9.531%), and traffic noise (3.746%). A visualization was created for the most common keywords determined in the study. The network visualization map for a total of 51 keywords processed from the minimum 10 keywords of all the articles is shown in Figure 4.

Most articles on noise pollution were published in the journal *Applied Acoustics* (58 articles; 3.196%). The 10 most active journals on this subject are shown in Table 4.

The most cited journals were mapped, by creating a citation source network visualization map. The minimum number for this visualization was taken as five articles (fig. 5).

DISCUSSIONS

The bibliometric analysis surveys the contributions of authors, countries, journals, and institutions in scientific studies, and can define the power of scientific impact by showing citations. Bibliometric software such as Gephi, Leximancer, VOSviewer, Scopus, and Web of Science has made it relatively easy to acquire large volumes of bibliometric data and enable the analysis of such data in a very pragmatic way, thereby raising scholarly interest in bibliometric analysis in recent years (17). In the current study, after the application of all inclusion and exclusion criteria, evaluations were made of 1815 articles published between 2001 and 2020 in the WoS database, and the findings and trends on noise pollution were presented. The study data included the number of publications by year, the number of citations, author, institution, journal, and country collaborations, the display of frequently used keywords, and the trend by year.

Table 2. Distribution of the number of publications, gross domestic product per capita and citation values by country.

Country	n (%)	n*	n**	TC	AC	H-index
United States	278 (15.317)	0.83	62.530	7958	28.63	45
China	263 (14.490)	0.19	16.117	3388	12.88	31
India	140 (7.713)	0.10	6.700	1330	9.5	20
England	137 (7.548)	2.42	46.659	4259	31.09	32
Spain	115 (6.336)	2.44	40.903	2395	20.83	26
Iran	110 (6.061)	1.28	12.389	714	6.49	15
Italy	97 (5.344)	1.55	42.492	1537	15.85	22
Germany	84 (4.628)	1.05	53.919	1976	23.52	25
Canada	76 (4.187)	2.00	49.031	1847	24.3	19
Australia	72 (3.967)	2.79	49.854	1031	14.32	19
Brazil	68 (3.747)	0.32	14.652	1140	16.76	19
Turkey	64 (3.526)	0.78	28.424	560	8.75	13
Netherlands	53 (2.920)	3.06	56.935	2383	44.96	21
France	52 (2.865)	0.76	46.184	1394	26.81	17
Romania	42 (2.314)	1.98	29.941	137	3.26	7
Scotland	36 (1.983)	6.62	46.659	698	19.39	16
Belgium	35 (1.928)	2.97	51.934	735	21	14
Pakistan	28 (1.543)	0.12	4.690	243	8.68	6
Greece	26 (1.433)	2.45	29.799	585	22.5	10
Poland	26 (1.433)	0.68	33.221	183	7.04	7
Singapore	25 (1.377)	4.26	97,341	325	13	9
South Korea	25 (1.377)	0.48	42.765	377	15.08	10
Switzerland	25 (1.377)	2.96	68.628	988	39.52	13
Japan	24 (1.322)	0.19	41.429	267	11.13	10
Malaysia	24 (1.322)	0.72	28.364	599	24.96	8
Portugal	24 (1.322)	2.34	34.894	373	15.54	10
Ireland	21 (1.156)	4.02	86.781	526	25.05	8

n* : number of articles per million population; n** : Gross domestic product per capita ($); TC: Total citations; AC: Average citations

Table 3. Top 10 high-frequency keywords.

Keywords	Frequency	%
Noise pollution	457	25.179
Noise	173	9.531
Traffic noise	68	3.746
Air pollution	57	3.140
Anthropogenic noise	56	3.085
Urbanization	38	2.094
Road traffic noise	37	2.036
Noise mapping	33	1.818
Pollution	33	1.818
Soundcape	31	1.708

Table 4. The top 10 journals of research on noise pollution.

Journals	Frequency	%
Applied Acoustics	58	3.196
Sustainability	31	1.708
Environmental Monitoring and Assessment	30	1.653
Journal of the Acoustical Society of America	28	1.543
Science of the Total Environment	27	1.488
Transportation Research Part D Transport and Environment	26	1.433
Marine Pollution Bulletin	24	1.322
International Journal of Environmental Research and Public Health	22	1.212
Noise Health	20	1.102
Sensors	20	1.102

There was determined to have been an increase in published articles in the last few years. It can be assumed that this will lead to the future publication of more papers on the research topic of noise pollution as an environmental and public health issue. The most cited articles spearhead the research topic. The authors of the top five most cited articles on noise pollution were Stansfeld SA, Miedema HME, Francis Clinton D, De Nazelle Audrey, and Rowe D. Bradley researchers should focus on these papers and the other most cited papers for further studies.

Although there were 109 countries publishing on noise pollution, the publication of 5 or more articles originated from only 55 countries.

The countries publishing the most articles were seen to be the USA, China, and India, in terms of articles per million population the leading countries were Scotland, Singapore, and Ireland, and in terms of the average number of citations, the top 3 countries were the Netherlands, Switzerland, and England. With their higher populations, China and India were ranked in the top three countries producing more articles in total was expected. The other country with the highest number of articles was the USA, which was not surprising as the USA is the leading country producing studies on many other topics (16, 18, 19). In addition to Scotland (GDP= $30,560), it was quite remarkable that Singapore (GDP=$97,341) and Ireland (GDP=$86,781) the two countries with the highest GDP per capita values, were the top 3 countries that published the most articles per million population.

This showed that there is a strong relationship between the level of economic development and the number of articles per million population within the main active countries. These relatively small countries had given weight to the publication of scientific articles. The relationship between the high-income level and the number of published articles has also been shown in previous studies (11, 13). In addition to traffic and urbanization, costly studies such as the creation of noise maps may have been effective in bringing developed countries to the fore on noise pollution. The Netherlands, Switzerland, and England are more cited due to their leading aspect in scientific studies as well as being developed countries and are considered to be the countries with the most impact per article. These countries are also European countries, and these European countries were able to demonstrate the importance of publication quality with the high numbers of average citations.

Traffic noise, road traffic noise, and urbanization were determined to be among the most used keywords, showing the effect of urbanization and traffic noise. In addition, the keywords of pollution, air pollution, and environmental noise indicate that noise is considered as an environmental problem. Anthropogenic noise keywords show that the effect of noise on the ecological natural environment and animals is a unique research area. The noise map keyword was one of the most used keywords, which demonstrated that noise mapping is also an important research area for noise pollution. However, creating a noise map for an area can be costly, so in developing or underdeveloped countries, in particular, R&D studies should be structured or accelerated to support global noise pollution scientific studies and the generation of noise maps.

Most articles on noise pollution were observed to be in *Applied Acoustics, Sustainability, Environmental Monitoring, and Assessment* journals but noise pollution articles in these journals constitute a relatively small proportion of all noise pollution articles (16-21). Although the top 10 journals that have published the most articles are shown in this study, it would be appropriate to recommend that researchers also review other journals.

This study presented a bibliometric analysis of noise pollution extracted from the Web of Science database. Although the study was as comprehensive, systematic, and objective as possible, there were also some limitations. The most important of the limitations was the use of only the WoS database. More comprehensive databases such as Pubmed, Google scholar, or Scopus could have been included. However, the WoS is conspicuous as the most widely used reliable database in bibliometric studies. Other limitations were that only articles in English were included, and searches at different times could increase the number of citations, especially for more recent articles. However, the study included articles that examined the effects of noise pollution not only on humans but also on animals. This subtle approach provided the opportunity to examine the effects of noise pollution more comprehensively.

CONCLUSIONS

1. The results of this study showed that there has been an increasing trend of articles on noise pollution in recent years.
2. The United States contributed the most publications, also the countries with the most articles per million population were Scotland, Singapore, and Ireland, respectively. The three most used keywords were “noise pollution”, “noise” and “traffic noise”.
3. This study can be considered to help researchers as it provides summary for current research. It was seen that the USA dominates research on noise pollution, and economically developed countries produce more articles per million population. There is a need for support for global noise pollution studies and for the generation of noise maps.

CONFLICT OF INTERESTS

The author declares no conflict of interest.

ETHICAL APPROVAL

The study conducted a bibliometric analysis of existing published article studies. This study did not require ethics committee approval.

REFERENCES

1. Gupta A, Jain K, Gupta S. Noise pollution and impact on children health. *The Indian Journal of Pediatrics.* 2018;85(4):300-306. doi:10.1007/s12098-017-2579-7
2. World Health Organization (2005). United Nations road safety collaboration: A handbook of partner profiles. Available from: http://apps.who.int/iris/bitstream/handle/10665/43159/9241592796.pdf;jsessionid=4F481A558B2E32C9E5B2036A995C5E92?sequence=1 [Accessed 5 Oct 2021].
3. Tao Y, Kou L, Chai Y, Kwan M-P. Associations of co-exposures to air pollution and noise with psychological stress in space and time: a case study in Beijing, China. *Environ Res.* 2020;110399. doi:10.1016/j.envres.2020.110399
4. Hunashal RB, Patil YB. Assessment of noise pollution indices in the city of Kolhapur, India. *Procedia-Social and Behavioral Sciences.* 2012;37:448-457. doi:10.1016/j.sbspro.2012.03.310
5. Rey Gozalo G, Barrigón Morillas JM. Analysis of sampling methodologies for noise pollution assessment and the impact on the population. *International journal of environmental research and public health.* 2016;13(5):490. doi:10.3390/ijerph13050490
6. De Kluizenaar Y, Gansevoort RT, Miedema HME, de Jong PE. Hypertension and road traffic noise exposure. *J. Occup. Environ. Med.* 2007;49:484-492. Available from: https://www.jstor.org/stable/44997545 [Accessed 5 Oct 2021].
7. WHO. (2012) Environmental health, inequalities in Europe World Health Organization WHO Regional Office for Europe, Denmark. Available from: https://www.euro.who.int/en/publications/abstracts/environmental-health-inequalities-in-europe.-assessment-report [Accessed 5 Oct 2021].
8. Murphy E, King EA, Rice HJ. Estimating human exposure to transport noise in central Dublin. *Ire*

land Environ Int. 2009;35:298-302. doi:10.1016/j.envint.2008.07.026
9. Ongel A, Sezgin F. Assessing the effects of noise abatement measures on health risks: A case study in Istanbul *Environmental Impact Assessment Review.* 2016;56:180-187. doi:10.1016/j.eiar.2015.10.008
10. Domer A, Korine C, Slack M, Rojas I, Mathieu D, Mayo A, Russo D. Adverse effects of noise pollution on foraging and drinking behavior of insectivorous desert bats. *MammalianBiology.* 2021;1-5. doi:10.1007/s42991-021-00101-w
11. Kiraz M, Demir E. A bibliometric analysis of publications on spinal cord injury during 1980-2018. *World Neurosurg.* 2020;136:e504-e513. doi:10.1016/j.wneu.2020.01.064
12. Lin CH, Chien TW, Yan YH. Predicting the number of article citations in the field of attention-deficit/hyperactivity disorder [ADHD] with the 100 top-cited articles since 2014: a bibliometric analysis. *Annals of General Psychiatry.* 2021;20(1):1-7. doi:10.1186/s12991-021-00329-3
13. Akyol A, Kocyigit BF. Ankylosing spondylitis rehabilitation publications and the global productivity: a Web of Science-based bibliometric analysis (2000-2019). *Rheumatology International.* 2021;1-8. doi:10.1007/s00296-021-04836-0
14. de Queiroz AP. Spatial analysis: a bibliometric approach (1950–2019). *Earth Science Informatics.* 2021;14(1):277-289. doi:10.1007/s12145-020-00546-6
15. Devos P, Menard J. Bibliometric analysis of research relating to hypertension reported over the period 1997-2016. *Journal of hypertension.* 2019;37(11):2116. doi:10.1097%2FHJH.0000000000002143
16. Akyol A, Kocyigit BF. Publication activity in the field of Sjögren's syndrome: a ten-year Web of Science based analysis. *Rheumatology International.* 2021; 41(4):763-769. doi:10.1007/s00296-020-04714-1
17. Donthu N, Kumar S, Mukherjee D, Pandey N, Lim WM. How to conduct a bibliometric analysis: An overview and guidelines. *Journal of Business Research.* 2021;133:285-296. doi:10.1016/j.jbusres.2021.04.070
18. Bulut E, Dokur M, Basar E. The Top 100 Cited Articles on Ocular Trauma: A Bibliometric Analysis. *Eye [globe].* 1997; 123(6). doi:10.5152/eurjther.2020.19115
19. Soteriades ES, Falagas ME. A bibliometric analysis in the fields of preventive medicine, occupational and environmental medicine, epidemiology, and public health. *BMC Public Health.* 2006; 6(1):1-8. doi:10.1186/1471-2458-6-301
20. Zhang J, Chen X, Gao X, Yang H, Zhen Z, Li Q et al. Worldwide research productivity in the field of psychiatry. *Int J Ment Health Syst.* 2017;11:20. doi:10.1186/s13033-017-0127-5
21. O'Neill CJ, Cassar-Gheiti AJ, Harty JA. Arthroplasty and global research output: a bibliometric analysis. *J Orthop.* 2019;17:187-192. doi:10.1016/j.jor.2019.06.017

Date of receipt of the manuscript: 23/11/2022
Date of acceptance for publication: 29/07/2023

BOOK REVIEWS AND PRESENTATIONS – REVUES DE LIVRES ET PRÉSENTATIONS

Recenzie la monografia colectivă *„Aspecte medicale ale schimbărilor climatice: realităţi şi perspective”*, autor coordonator: doctor în ştiinţe medicale, conferenţiar universitar Cătălina CROITORU

Monografia denumită „Aspecte medicale ale schimbărilor climatice: realităţi şi perspective", apărută în anul 2023 la Chişinău prin intermediul editurii "Print-Caro", se impune ca o lucrare ştiinţifică deosebit de aşteptată şi extrem de pertinentă. Abordează una dintre cele mai urgente probleme cu care se confruntă omenirea în prezent, subliniind conexiunile strânse dintre modificările climatice şi sănătatea umană. Această monografie reprezintă o resursă de nepreţuit pentru înţelegerea complexităţii şi implicaţiilor pe care schimbările climatice le au asupra sănătăţii omului şi pentru conturarea unor perspective fundamentate în abordarea acestei situaţii critice. Este un exemplu elocvent al implicării cercetătorilor în elucidarea problemelor contemporane de interes major, punând accentul pe necesitatea acţiunii urgente şi a strategiilor adecvate pentru a contracara impactul negativ al schimbărilor climatice asupra sănătăţii şi a promova un viitor mai durabil şi sănătos pentru generaţiile viitoare.

Intenţia conducerii editoriale a fost să creeze o lucrare comprehensivă şi informativă în domeniul schimbărilor climatice, cu scopul de a servi ca un instrument fundamental pentru specialiştii în medicină, medicii rezidenţi, studenţii universităţilor de medicină şi pentru publicul larg interesat de această temă crucială. Această monografie se doreşte a fi nu doar o sursă de cunoaştere, ci şi un ghid esenţial, oferind informaţii actualizate şi relevante pentru a înţelege impactul schimbărilor climatice asupra sănătăţii şi a pregăti cadrele medicale pentru adaptarea şi intervenţia adecvată în faţa acestor noi provocări. Este o iniţiativă menită să consolideze înţelegerea profundă a interacţiunilor complexe dintre mediul înconjurător şi sănătatea umană, subliniind importanţa abordării integrate şi a educaţiei continue în acest domeniu de interes global.

Monografia este rezultatul colaborării a 25 de autori, dintre care 4 coautori au provenit din România, aducând astfel o perspectivă internaţională şi un aport semnificativ la acest studiu amplu. Aceşti autori reprezintă domenii variate, cu expertiză în diferite instituţii, consolidând astfel diversitatea şi complexitatea abordărilor. Specialişti în medicină preventivă şi clinică aduc expertiză în efectele schimbărilor climatice asupra sănătăţii umane şi a tratamentelor asociate. Experţii în sănătate publică aduc o înţelegere profundă a impactului schimbărilor climatice asupra sănătăţii la nivel de comunitate şi politică de sănătate publică. Climatologii aduc în discuţie analizele şi proiecţiile climatice, contribuind la înţelegerea fenomenului schimbărilor climatice şi la anticiparea tendinţelor viitoare. Specialiştii în geonomie aduc cunoştinţe privind legăturile dintre mediu şi sănătate, contribuind la înţelegerea interacţiunilor complexe dintre factorii climatici şi sănătatea umană. Sociologii şi antropologii aduc în discuţie aspectele sociale şi culturale ale problemelor legate de schimbările climatice, evaluând percepţiile şi comportamentele oamenilor în acest context amplu. Această colaborare interdisciplinară adaugă o dimensiune semnificativă şi completă la monografie, oferind o abordare comprehensivă şi holistică a problemei schimbărilor climatice şi a impactului acestora asupra sănătăţii şi societăţii în ansamblu.

Scopul monografiei este să creeze o perspectivă unică, sensibilizând şi crescând gradul de conştientizare cu privire la problemele legate de schimbările climatice, aducând în prim-plan viziunile şi expertiza specialiştilor din domenii variate. Principalele aspecte detaliate în această lucrare cuprind:

- Fenomenul schimbărilor climatice: analiza fenomenului schimbărilor climatice la nivel global, european şi cu o focalizare specifică pentru Republica Moldova; dezbateri cu privire la încălzirea sau răcirea globală, susţinute de cercetări, studii şi soluţii, precum şi proiecţii climatice viitoare pe glob şi în Republica Moldova.
- Efectele schimbărilor climatice asupra sănătăţii: abordarea conceptului *One Health* şi evaluarea impactului climatic asupra patologiilor transmisibile şi netransmisibile; analiza implicaţiilor asupra agenţilor patogeni şi a vulnerabilităţilor, luând în considerare particularităţile diferitelor subpopulaţii (grupele de vârstă, gravide, diverse profesii).
- Adaptarea la schimbările climatice: investigarea mecanismelor de feedback la nivelul societăţii şi al sistemului de sănătate în contextul adaptării la schimbările climatice.
- Conştientizarea populaţiei privind riscurile pentru sănătate şi comunicarea climatică: evaluarea nivelului de conştientizare a populaţiei cu privire la riscurile pentru sănătate generate de schimbările climatice şi analiza strategiilor de comunicare eficiente.
- Migraţia climatică: examinarea aspectelor legate de migraţia populaţiei în contextul schimbărilor climatice şi a impactului asupra comunităţilor afectate.
- Educaţia şi instruirea climatică: abordarea educaţiei şi instruirii în domeniul schimbărilor climatice, evidenţiind importanţa sensibilizării şi a pregătirii adecvate a populaţiei şi a specialiştilor pentru a face faţă acestor provocări.

Monografia se concentrează asupra legăturilor esenţiale dintre încălzirea globală şi starea de sănătate a individului, subliniind influenţele reciproce asupra calităţii mediului şi a vieţii în general, precum şi consecinţele negative generate de intervenţia umană neechilibrată în natură şi degradarea acesteia. Autorii accentuează importanţa respectării legislaţiei şi implementării măsurilor menite să asigure puritatea elementelor de mediu şi să monitorizeze principalii indicatori de sănătate ai populaţiei.

Monografia pune în prim-plan necesitatea reflectării asupra numeroaselor probleme emergente şi a strategiilor de protecţie şi promovare a sănătăţii şi mediului. Conţinutul său corespunde cerinţelor actuale de instruire în domeniul sănătăţii publice, igienei şi medicinei, aliniindu-se recomandărilor internaţionale şi priorităţilor sistemului de educaţie medicală din Republica Moldova.

Această monografie, ca lucrare ştiinţifico-practică, îndeplineşte trei criterii majore: furnizarea de informaţii relevante şi actualizate, structurarea coerentă a subiectelor abordate şi orientarea cititorilor în privinţa strategiilor şi acţiunilor necesare. Este un ghid esenţial care contribuie la o înţelegere mai profundă a complexităţii problemelor legate de mediul înconjurător şi sănătatea umană, şi promovând adoptarea unor practici responsabile şi sustenabile.

Schimbările climatice pot fi văzute şi înţelese prin prisma unei calamităţi naturale deoarece au impacte considerabile asupra mediului şi a societăţii, adesea agravând şi intensificând evenimentele extreme şi dezastrele naturale. Prin această perspectivă, schimbările climatice reprezintă o ameninţare majoră şi persistentă pentru planeta noastră, având un impact profund asupra ecosistemelor, economiei şi calităţii vieţii umane. Abordarea lor necesită acţiuni globale imediate pentru a reduce emisiile de gaze cu efect de seră şi pentru a promova adaptarea şi sustenabilitatea, în vederea minimizării riscurilor asociate acestor calamităţi climatice.

Vasile DUMITRAŞ
doctor în ştiinţe medicale,
conferenţiar universitar, colonel medic (r),
Catedra de medicină militară şi a calamităţilor,
USMF „Nicolae Testemiţanu”, Republica Moldova

EVENTS/ANNIVERSARIES – EVENIMENTE/ANIVERSĂRI – ÉVÉNEMENTS/ANNIVERSAIRES – СОБЫТИЯ/ЮБИЛЕИ

JUBILEU DE AUR AL DOAMNEI GRETA BĂLAN: O POVESTE DE SUCCES ÎN CARIERA UNEI PROFESOARE

Educaţia este singura moştenire pe care o putem oferi generaţiilor viitoare.

Albert Einstein

O călătorie impresionantă în lumea cunoaşterii a început pentru doamna Greta Bălan în anul 1992, când a păşit pragul USMF „Nicolae Testemiţanu". A ales să urmeze calea medicinei preventive, o decizie ce i-a definit viitorul şi i-a pus în valoare pasiunea pentru sănătatea publică. În anii 1998-2000, a ales să-şi continue formarea şi activitatea profesională în cadrul Catedrei de microbiologie, virusologie şi imunologie, în căutarea unei înţelegeri profunde a acestor domenii esenţiale. Rezultatul acestor eforturi a fost obţinerea titlului de magistru în profilul „Microbiologie", o realizare care a atestat nu doar abilităţile ei academice, ci şi angajamentul pentru a se implica în studiul detaliat al microorganism elor şi imunităţii umane. Începând cu anul 2000 şi până în 2012, doamna Greta Bălan a desfăşurat o activitate susţinută în cadrul Catedrei de microbiologie şi imunologie, în calitate de asistent universitar. În anul 2008, a susţinut cu succes teza de doctor în ştiinţe medicale cu tema „Elaborarea şi evaluarea metodelor rapide pentru diagnosticul de laborator al infecţiilor tractului urinar". Între anii 2006 şi 2008, aprofundându-şi expertiza, a urmat programul de rezidenţiat cu specializarea în Microbiologi e. Din anul 2012 şi până în prezent, a continuat să-şi consolideze contribuţia în calitate de conferenţiar universitar şi şef de studii la Catedra de microbiologie, virusologie şi imunologie. În perioada 2014 - 2016, doamna Bălan şi-a extins orizonturile academice şi profesionale prin înrolarea în programul Şcolii de Management în Sănătate Publică. Acest demers a culminat cu obţinerea titlului de master în Managementul Sănătăţii Publice, dovedindu-şi astfel angajamentul faţă de învăţarea continuă şi dezvoltarea într-un context în continuă schimbare. În anul 2022 susţine cu brio teza de doctor habilitat în ştiinţe medicale „Compuşi noi cu acţiune asupra microorganismelor izolate din ulcere trofice", tot în acest an devine şefa Disciplinei de microbiologie şi imunologie. A publicat cca 200 de lucrări ştiinţifice şi metodico-didactice. Parcursul profesoarei Greta Bălan, de la înmatricularea plină de speranţă în 1992 până la realizările sale în microbiologie şi imunologie, ilustrează frumuseţea căutării continue a cunoaşterii şi puterea de transformare a educaţiei într-o forţă motrică pentru schimbare şi progres.

Mulţi ani prosperi, Doamnă Greta BĂLAN!

Cu profund şi deosebit respect, consiliul de redacţie al Revistei *One Health & Risk Management*

VICTORIA BUCOV - PERSONALITATE NOTORIE ÎN DOMENIUL SĂNĂTĂȚII PUBLICE

Două calități pe lume nu pot fi simulate
și nici înlocuite: inteligența și bunătatea.

(Ileana Vulpescu)

O figură proeminentă a serviciului de sănătate publică din Republica Moldova, reprezentativă pentru istoria Agenţiei Naţionale pentru Sănătate Publică – este cea a profesorului-cercetător Victoria Bucov, epidemiolog şi imunolog, şefă a Laboratorului ştiinţific „Supravegherea rezistenţei antimicrobiene”.

Fiecare om care vine pe acest pământ are o menire, care îl ghidează, îi marchează viaţa şi activitatea profesională. Uneori, omul singur îşi modelează soarta, aşa cum a făcut-o şi dna Victoria Bucov. Fiind un model al modestiei, Dumneaei, totodată şi-a stabilit scopuri ambiţioase, realizate prin perseverenţă, dedicaţie, muncă asiduă, transformând năzuinţele în succese frumoase, demne de toată admiraţia.

Este o persoană care nu oboseşte să muncească zi de zi, cucerind noi înălţimi ale profesionalismului. Fiind o doamnă de o moralitate aleasă, dna Victoria Bucov emană mereu energie spirituală şi în sfera activităţii profesionale, devenind aproape un simbol al tactului, respectului, delicateţei şi a bunelor maniere.

Cu o capacitate de de muncă inestimabilă şi un spirit mereu în căutare, doamna Profesor devine o personalitate proeminentă, un savant, care a creat o moştenire inestimabilă, înscrisă atât în lucrările sale, cât şi a discipolilor şi colegilor, pe care îi ghidează în drumul spre cunoaştere.

Sănătatea societăţii, în care trăim, mai depinde şi de modul în care construim şi fortificăm sistemul de supraveghere a sănătăţii publice. La acest capitol, dna Bucov, prin rezultatele cercetărilor ştiinţifice realizate, a argumentat necesitatea de a fi introduse în programele naţionale de imunizare - vaccinurile, care previn probleme serioase de sănătate: boli infecţioase, dizabilitate sau chiar decese. Efortul echipei conduse de către dna Bucov a rezultat în măsuri specifice de prevenire a bolilor infecţioase şi vaccinarea a zeci de mii de copii şi maturi, cu biopreparate sigure şi eficiente.

Ajunsă la această treaptă a experienţei profesionale, profesorul Victoria Bucov consideră că, la baza unui destin împlinit stau responsabilitatea, onestitatea, interesul, devotamentul, voinţa. Aceste principii sunt aplicate în permanenţă în activitatea pe care o desfăşoară zi de zi.

Mult stimată dnă Victoria Bucov, cu ocazia frumosului jubileu, ne exprimăm înalta noastră consideraţie şi gratitudine, şi Vă aducem sincere urări de bine, multă sănătate, cu realizări frumoase în îndeplinirea tuturor dorinţelor!

Mulţi ani prosperi, Doamna Victoria BUCOV!

Cu profund şi deosebit respect,
colegii consiliului de redacţie al Revistei ştiinţifice *One Health & Risk Management*

REQUIREMENTS FOR AUTHORS

Rules of drafting
The manuscript (written in English and French) should be in accordance with the guidelines published in: *Uniform Requirements for Manuscripts Submitted to Biomedical Journal (1994) Lancet 1996, 348, V2; 1-4* (www.icmje.org). The manuscripts should be written in font Cambria, size 11 points, spaced at 1.0, fully justified alignment, fields 2 cm on all sides. All pages must be numbered consecutively (in the right bottom corner) and continuously. Abbreviations should be explained at first occurrence in the text and should not be excessively used. The manuscripts must not exceed the number of words (without the title, affiliation, abstract and references): review articles – 4,500 words; research articles – 3,000 words; expert opinions – 2,500 words; case presentation – 1,700 words; experimental and clinical notes – 1,300 words; book reviews and presentations – 2,000 words; teaching articles – 4,000 words. The volume of tables and figures should not exceed ⅓ from the volume of the manuscript. The journal reserves the right to make any other formatting changes. Rejected manuscripts are not returned.

All manuscripts submitted for publication should be accompanied by two abstracts: in the language of origin of the article and English.

Title and authors
The title should be as short as possible (maximum – 120 signs with spaces), relevant for the manuscript content. The names of the authors should be written in full: name, surname (*e.g.:* Jon JONES). Affiliation should include: Department/Unit/Chair, University/Hospital, City, Country of each author. Beneath the affiliation, the author`s details and contact information – e-mail address (*e.g.:* corresponding author: Jon Jones, e-mail: jon.jones@gmail.com).

The structure of the manuscript
The manuscript should comprise the following subheadings (capitalized):

- **SUMMARY**
- **INTRODUCTION** (will reflect the topicality and the general presentation of the problem studied, purpose and hypothesis of the study)
- **MATERIAL AND METHODS**
- **RESULTS**
- **DISCUSSIONS**
- **CONCLUSIONS**
- **CONFLICT OF INTERESTS**
- **ACKNOWLEDGEMENT** (optional)
- **ETHICAL APPROVAL** (specify the presence or absence of a positive opinion from the ethics committee: no, date, institution ad informed consent)
- **REFERENCES**

The **summary** should contain 1,600 signs with spaces:

- **Introduction**
- **Material and methods**
- **Results**
- **Conclusions**
- **Key words:** 3-5 words

The summary should not include tables, charts, and bibliographic notes; information not included in the article.

Figures. The text included in figures should be written in font Cambria, 10 point. Each figure should be accompanied by a heading and legend. They should be numbered with Arabic numerals and placed in parentheses (*e.g.:* fig. 1). Both the title (*e.g.* Figure 1) and legend are centred, below the figure.

Tables. The text included in tables should be written in font Cambria, 10 point. Each table should be accompanied by a heading. Tables should be inserted into the text and adjusted to the width of the page. The tables are numbered in Arabic numerals and mentioned in body text in parentheses (*e.g.* tab. 1). The title of the table is centred on the top of the table (*e.g.* Table 1).

References are numbered in the order they appear in the paper. The reference sources are cited at the end of the article by using AMA style and will include only the references cited within the text (the reference is numbered within round parentheses). The in-text citations that appear more than once are numbered si-milarly as in the first citation. The number of references should not exceed 50 sources. The scientific authors are responsible for the accuracy of their writings. The reference list should include only those references that have been consulted by the authors of the manuscript. The elements of the reference sources are written exactly in accordance with the requirements.

For more information see: http://journal.ohrm.bba.md/index.php/journal-ohrm-bba-md/editing_guidelines

CERINȚE PENTRU AUTORI

Reguli de tehnoredactare
Pregătirea manuscrisului (elaborat în limbile engleză și franceză) va fi în conformitate cu instrucțiunile publicate în: *Uniform Requirements for Manuscripts Submitted to Biomedical Journals (1994) Lancet 1996, 348, V2; 1-4* (www.icmje.org). Manuscrisele trebuie să fie cu font Cambria, dimensiune 11 puncte, spațiat la interval 1,0, aliniere justificată, câmpurile 2 cm pe toate laturile. Toate paginile trebuie să fie numerotate consecutiv (în colțul de jos, în partea dreaptă) și să includă nume-rotarea continuă a paginilor. Abrevierile trebuie să fie explicate la prima apariție în text și nu trebuie utilizate excesiv. Manuscrisele nu trebuie să depășească (fără a număra titlul, afilierea, rezumatul și refe-rințele): pentru articole de sinteză/referate – 4500 de cuvinte; pentru articole de cercetare – 3000 de cuvinte; pentru opinii ale experților – 2500 de cuvinte; prezentare de caz și imagini din practica clinică/laborator – 1700 de cuvinte; note experimentale și clinice – 1300 de cuvinte; recenzii și prezentări de carte – 2000 de cuvinte; articole didactice – 4000 de cuvinte. Volumul tabelelor și figurilor nu trebuie să depășească ⅓ din volumul manuscrisului. Revista își rezervă dreptul de a face orice alte modificări de formatare. Manuscrisele respinse nu sunt returnate.

Toate manuscrisele transmise spre publicare trebuie să fie însoțite de două rezumate: în limba de origine al articolului și în limba engleză.

Titlul și autorii
Titlul ar trebui să fie cât mai scurt posibil (maximum - 120 de semne cu spații), elocvent pentru conținutul manuscrisului. Numele autorilor vor fi scrise deplin: prenume, nume de familie (*ex:* Ion RUSU). Afilierea va include: Secția/Departamentul/Catedra, Universitatea/Spitalul, Orașul, Țara pentru fiecare autor. Se vor menționa obligatoriu, mai jos, datele autorului corespondent și informațiile de contact – adresa de e-mail (*ex:* autor corespondent: Ion Rusu, e-mail: ion.rusu@gmail.com).

Structura manuscrisului
Manuscrisul va cuprinde următoarele subtitluri (scrise cu majuscule):

- **REZUMAT** (vezi cerințele mai jos)
- **INTRODUCERE** (se va reflecta actualitatea și prezentarea generală a problemei studiate, scopul și ipoteza studiului)
- **MATERIAL ȘI METODE**
- **REZULTATE**
- **DISCUȚII**
- **CONCLUZII**
- **CONFLICT DE INTERESE**
- **MULȚUMIRI ȘI FINANȚARE** (optional)
- **APROBAREA ETICĂ** (se va specifica prezența sau lipsa avizului pozitiv de la comitetul de etică: nr, data, instituția și acordul informat)
- **REFERINȚE**

Rezumatul va conține până la 1600 de semne cu spații și va cuprinde:

- **Introducere**
- **Material și metode**
- **Rezultate**
- **Concluzii**
- **Cuvinte cheie**: 3-5 cuvinte

În rezumat nu vor fi incluse tabele, grafice și note bibliografice; informații care nu sunt prezentate în studiu.

Figuri. Textul inclus în figuri trebuie să fie scris cu font Cambria, dimensiune 10 puncte. Fiecare figură trebuie să fie însoțită de titlu și legendă. Ele vor fi numerotate cu cifre arabe și vor fi menționate în text în paranteze (*ex:* fig. 1). Titlul (*ex:* Figura 1) și legenda figurii trebuie să fie scrisă centrat, sub figură.

Tabele. Textul inclus în tabele trebuie să fie scris cu font Cambria, dimensiune 10 puncte. Fiecare tabel trebuie să fie însoțită de titlu. Tabelele vor fi inserate în text, fără a depăși lățimea unei pagini. Ele vor fi numerotate cu cifre arabe și vor fi menționate în text în paranteze (*ex:* tab. 1). Titlul tabelului va fi poziționat deasupra tabelului centrat (*ex:* Tabelul 1).

Referințele trebuie să fie numerotate în ordinea apariției în text. Citarea sursei de referință va fi conform stilului *AMA*, plasată la sfârșitul articolului și va include doar referințele citate în text (menționând numărul de referință în paranteză rotundă). Dacă aceeași referință este citată de mai multe ori, ea va fi trecută în text cu același număr ca la prima citare. Numărul total de referințe nu va depăși 50 de surse. Acuratețea datelor ține de responsabilitatea autorului.

Pentru mai multe informații consultații: http://journal.ohrm.bba.md/index.php/journal-ohrm-bba-md/editing_guidelines

EXIGENCES POUR LES AUTEURS

Normes de rédaction
La préparation des manuscrits (rédigés en anglais et français) sera conforme aux instructions publiées dans *Uniform Requirements for Manuscripts Submitted to Biomedical Journals (1994) Lancet 1996, 348, V2 ; 1-4* (www.icmje.org). Les manuscrits doivent être en police Cambria, taille 11 points, espacés à l'intervalle 1,0, alignement justifié, champs 2 cm de tous les côtés. Toutes les pages doivent être numérotées consécutivement (dans le coin inférieur droit) et inclure une numérotation continue des pages. Les abréviations doivent être expliquées lors de la première apparition dans le texte et ne doivent pas être utilisées de manière excessive. Les manuscrits ne doivent pas dépasser (sans mentionner le titre, l'affiliation, le résumé et la bibliographie) le vo-lume suivant: pour articles de synthèse/rapports – 4500 mots; pour les articles de recherche – 3000 mots; pour les opinions d'experts – 2500 mots; présentation de cas et photos de la pratique clinique/de laboratoire – 1700 mots; notes expérimentales et cliniques – 1300 mots; commentaires et présentations de livres – 2000 mots; articles pédagogiques – 4000 mots. Le volume des tableaux et des figures ne doit pas dépasser ⅓ du volume du manuscrit. La revue se réserve le droit d'apporter toute autre modification de formatage. Les manuscrits rejetés ne sont pas retournés.

Tous les manuscrits à publier doivent être accompagnés par deux résumés: dans la langue originale et en anglais.

Titre et auteurs
Le titre doit être le plus court que possible (maximum – 120 signes avec espaces), éloquent pour le contenu du manuscrit. Les noms des auteurs seront écrits complets: prénom, nom (*ex:* Albert LEBRUN). Quant à l'affiliation, on devra indiquer: Section/ Département/Chaire, Université/Hôpital, Ville, Pays – pour chaque auteur. Les données de l'auteur correspondant et les coordonnées – adresse e-mail (*ex:* auteur correspondant: Albert Lebrun, e-mail: albert.lebrun@gmail.com*)* seront obligatoires ci-dessous.

Structure du manuscrit
Le manuscrit comprendra les sous-titres suivants (avec lettres majuscules):

- **RÉSUMÉ** (voir les exigences ci-dessous)
- **INTRODUCTION** (reflétera l'actualité et la présentation générale du problème étudié, le but et l'hypothèse de l'étude)
- **METHODES**
- **RESULTATS**
- **DISCUSSIONS**
- **CONCLUSIONS**
- **CONFLIT D'INTERETS**
- **REMERCIEMENTS ET FINANCEMENT**
- **APPROBATION ÉTHIQUE** (préciser la présence ou l'absence d'avis favorable du comité d'éthique: no, date, institution et consentement éclairé)
- **REFERENCES**

Le **résumé** contiendra 1600 signes avec espaces:

- **Introduction**
- **Méthodes**
- **Résultats**
- **Conclusions**
- **Mots clés**: 3-5mots.

Le résumé ne comprendra pas des tableaux, graphiques et des notes bibliographiques; des informations non présentées dans l'étude.

Figures. Le texte inclus dans les figures doit être écrit avec police Cambria, taille 10 points. Chaque figure doit être accompagné par un titre et une légende. Ceux-ci seront numérotés avec des chiffres arabes et mentionnés dans le texte entre parenthèses (*ex:* fig. 1). Le titre (*ex:* Figure 1) et la légende de la figure doivent être centrés, au-dessous de la figure.

Tableaux. Le texte inclus dans les tableaux doit être écrit avec police Cambria, taille 10 points. Chaque tableau doit être accompagné par un titre. Les tableaux seront numérotés avec des chiffres arabes, mentionnés dans le texte entre parenthèses (*ex:* tab. 1), et seront insérés dans le texte, sans dépasser la largeur d'une page. Le titre du tableau sera placé au-dessus du tableau, centré (*ex:* Tableau 1).

Les **références** doivent être numérotées dans l'ordre où elles apparaissent dans le texte. La citation de la source de référence sera de style *AMA*, placée à la fin de l'article et n'inclura que des références citées dans le texte (mentionnant le numéro de référence entre parenthèses rondes). Si la même référence est citée plusieurs fois, elle sera transmise dans le texte avec le même numéro que celui de la première citation. Le nombre total de références ne dépassera pas 50 sources. La responsabilité pour l'exactitude des données est à la charge de l'auteur. Il faut indiquer dans le manuscrit seulement les références vraiment consultées par les auteurs. Les composants des sources de référence doivent être rédigés strictement selon les exigences.

Pour plus d'informations, voir: http://journal.ohrm.bba.md/index.php/journal-ohrm-bba-md/editing_guidelines

ТРЕБОВАНИЯ ДЛЯ АВТОРОВ

Правила составления

Подготовка рукописи (разработанной на английском и французском языках) будет осуществляться в соответствии с инструкциями, опубликованными в: *Uniform Requirements for Manuscripts Submitted to Biomedical Journals (1994) Lancet 1996, 348, V2; 1-4* (www.icmje.org). Авторы должны использовать шрифт Cambria, размер 11 точек, с интервалом 1,0, выравнивание по ширине, поля 2 см со всех сторон. Все страницы должны быть пронуме-рованы последовательно (в правом нижнем углу) и включать непрерывную нумерацию страниц. Сокращения должны быть объяснены при первом появлении в тексте и не должны использоваться чрезмерно. Объем рукописей не должен превышать (без названия, принадлеж-ности, резюме и литературы): для обзорных статей/рефератов – 4500 слов; для научных ста-тей – 3000 слов; для экспертных заключений – 2500 слов; для презентации случаев из клини-ческой/лабораторной практики – 1700 слов; для экспериментальных и клинических заметок – 1300 слов; для рецензий и презентаций книг – 2000 слов; для учебных статей – 4000 слов. Объем таблиц и рисунков не должен превышать ⅓ от объема рукописи. Журнал оставляет за собой право вносить любые другие изменения форматирования. Отклоненные рукописи не возвращаются.

Все рукописи, представленные для публикации, должны сопровождаться двумя резюме: на языке оригинала статьи и на английском языке.

Название и авторы

Название должно быть как можно короче (максимум – 120 знаков с пробелами), но дос-таточно информативным для содержания рукописи. Фамилии авторов будут написаны полностью: имя, фамилия (*например:* Иван ИВАНОВ). Принадлежность будет включать: Отделение/ Департамент/Кафедра, Университет /Больница, Город, Страна для каждого автора. Данные соответствующего автора и контактная инфор-мация – адрес электронной почты (*например:* контактная информация: Иван Иванов. e-mail: ivan.ivanov@gmail.com) будут обязательно ниже.

Структура Рукописи

Рукопись будет включать в себя следующие подзаголовки (они должны быть заглавными):

- **РЕЗЮМЕ** (см. требования ниже)
- **ВВЕДЕНИЕ** (будет отражать актуаль-ность и общее представление изучаемой проблемы, цель и гипотезу исследования)
- **МАТЕРИАЛЫ И МЕТОДЫ**
- **РЕЗУЛЬТАТЫ**
- **ДИСКУССИИ**
- **ВЫВОДЫ**
- **КОНФЛИКТ ИНТЕРЕСОВ**
- **БЛАГОДАРНОСТИ И ФИНАНСИРОВАНИЕ**
- **ЭТИЧЕСКОЕ ОДОБРЕНИЕ** (указать наличие или отсутствие одобрения со стороны комитета по этике: №, дата, учреждение и информированное согласие)
- **ЛИТЕРАТУРА**

Резюме должно содержать 1600 знаков с пробелами и будет включать в себя следующие подзаголовки:

- **Введение**
- **Материалы и методы**
- **Результаты**
- **Выводы**
- **Ключевые слова:** 3-5 слов

Резюме не должно включать таблицы, диаграммы и библиографические заметки, инфор-мацию, не представленную в исследовании.

Рисунки (графики, диаграммы). Текст, включенный в рисунки, должен быть написан в Cambria, размер 10 пунктов. Каждый рисунок должен сопровождаться заголовком и описанием. Название (*например:* Рисунок 1) и описание рисунка должны быть вписаны по центру, в низу рисунка. Они должны быть пронумерованы арабскими цифрами и указаны в тексте в скобках (*например:* рис. 1).

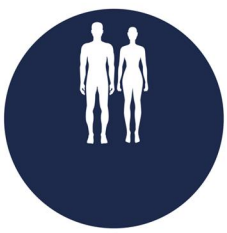
Таблицы. Текст, включенный в таблицы, должен быть написан в Cambria, размер 10 пунктов. Каждая таблица должна сопровождаться заголовком. Они должны вставляться в текст, не превышая ширину страницы. Должны быть пронумерованы арабскими цифрами и указаны в тексте в скобках (*например:* таб. 1). Название таблицы должно располагаться над таблицей в центре (*например:* Таблица 1).

Литература. Источники должны быть пронумерованы в порядке их появления в тексте. Ссылки на источники должны быть в стиле *AMA*, помещены в конце статьи и включать только источники, цитируемые в тексте (упоминание номера источника в круглых скобках). Если один и тот же источник цитируется несколько раз, он будет передан в тексте с тем же номером, что и первый раз. Общее количество источников не должно превышать 50. Ответственность за точ-ность данных лежит на авторе. Будут цитиро-ваться только те источники, с которыми ознакомились авторы рукописи. Компоненты справочных источников должны быть написаны строго в соответствии с требованиями.

Дополнительная информация на: http://journal.ohrm.bba.md/index.php/journal-ohrm-bba-md/editing_guidelines

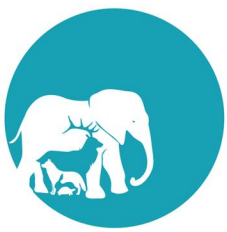
The *One Health* concept

Human health



The WHO defined health in 1946 as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity", with the later addition of "the capacity to lead a socially and economically productive life".

Animal health



The OIE defines animal welfare in 2008: an animal is in good condition if it is healthy, enjoys comfort, is well fed, is safe, is able to display its innate (natural) behavior and does not suffer from unpleasant conditions such as pain, fear and stress.

Plant and environmental health

Environmental health refers to those aspects of human health that include the quality of life determined by physical, biological, socio-economic and psycho-social factors in the environment. The interrelationships of people with the environment concern medicine, when an ecological system is in a state of equilibrium, the health of the population prevails.

Globally, the *One Health* concept is a worldwide strategy to expand interdisciplinary collaborations and communications in all aspects related to the health care of humans, domestic animals or wildlife, which can no longer be approached separately, but only jointly.

One Health addresses not only human and animal disease concerns, but also issues related to lifestyle, diet, exercise, the impact of different types of human-animal relationships, and environmental exposures that can affect both populations. In order to achieve the expected effects, it is also necessary to educate the population to make them aware of the risk factors and benefits of prevention, as well as communication and understanding between patients and healthcare providers.


WORLD of JOURNALS

DOAJ
DIRECTORY OF OPEN ACCESS JOURNALS

CORE

Academic Resource Index
ResearchBib

НАУЧНАЯ ЭЛЕКТРОННАЯ БИБЛИОТЕКА
eLIBRARY.RU

INTERNET ARCHIVE

OCLC
WorldCat®

DRJI
Directory of Research Journals Indexing

We are Crossref
Member

Instrumentul Bibliometric Național
www.ibn.idsi.md

BȘM

I2OR

EuroPub

Google Scholar

OpenAIRE

CYBERLENINKA

ROAD

CiteFactor
Academic Scientific Journals

zenodo