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Microbiological analysis of wound content in patients with type 2 diabetes mellitus with diabetic foot syndrome

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Abstract. An important component of the comprehensive conservative treatment of diabetic ulcers is antibacterial therapy, as without timely and correct treatment, patients may develop toxic shock syndrome, leading to multiple organ failure. The aim of the study was to examine the species variety of the wound content in diabetic foot ulcers in patients with type 2 diabetes and to determine the susceptibility of the isolated microorganisms to antimicrobial drugs. Given the results of the bacteriological examination of the wound content, the most frequently isolated microorganisms were: *Staphylococcus aureus, Staphylococcus haemolyticus, Klebsiella* spp., *Pseudomonas aeruginosa, Corynebacterium* spp., *Escherichia coli, Proteus* spp., and fungi of the genus *Candida* spp. The study not only investigated the microbial variety in diabetic foot syndrome but also established the effectiveness of applying antibacterial agents to specific isolated pathogens.

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The most effective antibiotics were tigecycline and vancomycin, with the susceptibility of the specified microorganisms ranging from 89.6% to 100.0%, respectively. The susceptibility of *S. aureus* and *S. haemolyticus* to amikacin was 75.9% and 62.1%, respectively. *P. aeruginosa* was susceptible to only 3 out of the 12 antibiotics included in the study, namely: amikacin, vancomycin, and ciprofloxacin. *Klebsiella* spp. showed moderate susceptibility (45.5%) only to doxycycline. *Corynebacterium* spp. was resistant to most of the studied antimicrobial agents, and only to amikacin, tigecycline, vancomycin, and chloramphenicol, the susceptibility level was within 50.0-100.0%. *E. coli* demonstrated high susceptibility (100.0%) to such antimicrobial drugs as ciprofloxacin, ceftazidime, chloramphenicol, amikacin, azithromycin, tigecycline, and ertapenem. *Proteus* spp. was susceptible (100.0%) to chloramphenicol, amikacin, azithromycin, tigecycline, and ertapenem. The identification of the microbial diversity of the wound contents of a diabetic ulcer and a broad antibiotic profile will allow the optimization of antibiotic therapy in accordance with the treatment protocol for this pathology and prevent the development of antibiotic resistance

Keywords: metabolic disease; diabetic foot infection; microorganisms; antibiotics; antibiotic resistance

Introduction

Type 2 diabetes mellitus (T2DM) is considered a complex healthcare problem and ranks among the leading diseases in modern society. Since 2000, due to its high rate of spread and numerous complications, T2DM has raised concerns not only in Ukraine but also internationally [1]. According to the World Health Organization (WHO), about 500 million people suffer from diabetes today. Scientists predict that by 2040, the number of cases will exceed 400 million [2, 3]. In Ukraine, there are 3.5 million people with diabetes, with 1.23 million diagnosed cases (35.0% of all people with diabetes) [1, 2]. Monitoring studies show that the number of people with diabetes in Ukraine is increasing by an average of 5.0-7.0% annually [1].

One of the most common and severe surgical complications of type 2 diabetes is diabetic foot syndrome (DFS), which complicates the course of the disease in nearly 30.0-80.0% of cases and is the leading cause of non-traumatic amputations. According to the global registry and medical statistics in developed European countries, the annual incidence of DFS is 23.0-25.0% among diabetic patients and is the cause of hospitalization for every fourth patient, with one in seven undergoing high limb amputation [4, 5]. According to O. Laktionova *et al.* [2], the recurrence rate can reach 45.0%. DFS is a complex of symptoms that develop pathological changes in the feet, such as purulent-necrotic processes, bone and joint lesions, and ulcers, arising against the background of specific changes in peripheral nerves, blood vessels, skin, and soft tissues [5, 6].

Diabetic foot syndrome is considered a major medical and social problem for both patients and healthcare systems not only in Ukraine but worldwide. The prevalence of chronic wound defects in the soft tissues of the lower extremities ranges from 4.0 to 15.0%. Among all hospitalised individuals with diabetic foot, patients with trophic foot ulcers constitute 6.0-10.0%, and their hospital stay is 60.0% longer compared to patients without skin integrity violations [6, 7].

The analysis of numerous studies demonstrates that the cause of mild to moderate ulcers is predominantly gram-positive bacteria. At the same time, severe or chronic forms of infection are more often characterised by a polymicrobial nature. Among gram-positive bacteria, staphylococci (*Staphylococcus aureus, Staphylococcus epidermidis*) are most commonly isolated. *Hemolytic streptococci* are verified much less frequently. Gram-negative aerobic bacteria are usually represented by microorganisms from the *Enterobacteriaceae* group and *Pseudomonas* spp. [8, 9].

There is often a problem associated with the ineffectiveness of existing treatment protocols for this pathology. Scientists note that this situation may be due to the presence of Methicillin-resistant *Staphylococcus aureus* (MRSA), which leads to the complication of the purulent process and increased mortality, including among patients with diabetic foot syndrome [10]. Anaerobic bacteria are considered the main pathogens, mostly in patients with ischaemic forms of DFS or gangrene. They account for up to 50.0% of all isolated strains. There are suggestions that *Corynebacterium* spp. may play a pathogenic role around necrotic tissues [8, 11].

S. Shapoval *et al.* [10] have shown that the following microorganisms can also be isolated among the mentioned bacteria in purulent-necrotic processes: *Enterococcus faecalis, Enterococcus faecium, Streptococcus agalactiae*, and *Kocuria kristinae*. In turn, A. Prevar *et al.* [12] show in their work the presence of *Citrobacter freundii*, *Enterobacter cloacae*, *E. aerogenes, S. viridians,* and *S. agalactiae*. Therefore, it is crucial to establish the role of microorganisms in the development and course of such purulent-necrotic processes, as well as the rationality of antibiotic therapy. The purpose of the study was to investigate the species variety of the wound content of diabetic foot ulcers in type 2 diabetes and to determine the susceptibility of the isolated microorganisms to antimicrobial drugs.

Materials and Methods

The study involved 80 patients with T2DM and DFS aged 45 to 75 years who were treated at the Municipal Non-Profit Enterprise "Ternopil City Emergency Hospital" from February to October 2023. The inclusion criteria were: people of both sexes, people aged over 18, verified diagnosis of type 2 diabetes mellitus, diabetic foot syndrome and consent to participate in the study. The exclusion criteria were: chronic diseases in the acute phase as well as in the phase of decompensation, treatment with glucocorticosteroids, pregnancy, mental disorders, cancer and suspected cancer, refusal to

participate in the study. All patients were diagnosed with type 2 diabetes: 93.3% were in the subcompensation stage and 6.7% in the decompensation stage. Patients were admitted with pronounced purulent-necrotic lesions in various areas of the foot, with a mixed form of DFS. All patients signed an informed consent to participate in the study.

All patients included in the study were indicated for surgical intervention due to diabetic foot syndrome complicated by purulent-necrotic lesions. To prescribe rational antibiotic therapy, all patients underwent bacteriological examination of purulent discharge from the wounds with microbiota verification. For this purpose, the collection of biological material (pus, exudate, wound content) from the wound surfaces was carried out under aseptic conditions using sterile swabs before the use of antibacterial drugs (ABD). The skin around the wound edges was pre-treated with 70.0% alcohol. The material for the study was taken from the centre to the periphery of the wound surface using two sterile swabs. The first swab was used to prepare a smear, which was stained using the Gram method. The second swab was used to inoculate clinical material onto the surface of solid nutrient media for bacteriological analysis.

To identify microorganisms, the material was cultured on the following nutrient media: for aerobic bacteria – blood agar, yolk-salt agar, sugar broth, serum agar, Endo medium for enterobacteria (Biolife Italiana S.r.l.); for anaerobic bacteria – Wilson-Blair medium, and thioglycolate broth, using gas-generating box to create anaerobic conditions – GENboxanaer (BioMerieux, France). The cultures were incubated in a thermostat at 37°C for 24-48 hours. For the detection of fungi of the genus *Candida* spp., Sabouraud medium (FARMAKTIV LLC, Kyiv, Ukraine) was used with subsequent incubation in a thermostat at 27-30°C for 5 days. The identification of microorganisms was based on their morphological, tinctorial, and cultural characteristics.

To determine the susceptibility of the isolated pathogenic microorganisms to antibacterial drugs, the Kirby-Bauer qualitative method was used with standard discs. Pure bacterial cultures were cultivated on the Mueller-Hinton medium, and fungi on Sabouraud medium. The following antibiotics were tested in the study: ciprofloxacin, ceftazidime, chloramphenicol, amikacin, azithromycin, ceftriaxone, doxycycline, erythromycin, methicillin, tigecycline, vancomycin, and ertapenem. The assessment and analysis of the results were conducted by determining the diameters of growth inhibition zones according to standard table data [13].

Statistical processing of the numerical data was performed using Excel software (Microsoft, USA) tabulating the data and expressing them in per cents. All studies were conducted in compliance with the main bioethical norms and the requirements of the Helsinki Declaration, cited by O. Nawrot [14], as confirmed by the conclusion of the Bioethics Commission of I. Horbachevsky Ternopil National Medical University, Ministry of Health of Ukraine (protocol No. 77 dated April 18, 2024).

Results

Pathological material for determining the species diversity of microorganisms causing and promoting limb suppuration in diabetes was obtained from patients, among whom the share of men was 66.25% and women 33.75% (see Table 1).

Table 1. Age distribution of patients with T2DM and DFS

Age	Women %		Men %	
	n	%	n	%
Until 50 years old	6	22.20	12	22.64
51-60	6	22.20	13	24.52
61-70	10	37.03	13	24.52
After 70	5	18.51	15	28.30
In total	27	33.75	53	66.25

Source: table compiled by the authors

According to the results of the bacteriological study of the wound content, the following microorganisms were identified: *Staphylococcus aureus, Staphylococcus haemolyticus, Klebsiella* spp., *Pseudomonas aeruginosa, Corynebacterium* spp., *Escherichia coli, Proteus* spp., and fungi of the genus *Candida* spp. With high frequency (30.0%), *S. aureus* and *S. haemolyticus* were found (see Fig. 1).

This percentage of identification of the specified microorganisms is due to their properties: the ability to produce proteolytic factors that disrupt the skin barrier. Therefore, staphylococci are the main colonisers of chronic suppurative wounds. Next in prevalence in the microbiome of purulent wounds against the background of diabetes was the gram-negative rod *Klebsiella* spp., with a frequency of isolation among bacteria of 11.0%. Microbiological examination of ulcers in DFS showed the presence of such bacteria in the biomaterial as *Corynebacterium* spp. These organisms are part of the normal microbiota of mucous membranes and healthy skin, especially in areas like the feet, and are considered colonizing rather than pathogenic bacteria. The frequency of detection of corynebacteria was 9.0%.

Results of microbiological research: *Pseudomonas* spp., *E. coli,* and *Proteus* spp. were identified significantly less frequently, with less than 10.0% occurrence: *P. aeruginosa* – 6.0%, *E. coli* – 5.0%, and *Proteus* spp. – 5.0%. Typically, *P. aeruginosa* is rarely verified in purulent-necrotic lesions. In 4.0% of cases, fungi of the genus *Candida* spp. were isolated in addition to the microorganisms described above. Their appearance is possibly related to the immunosuppression associated with T2DM (see Fig. 1).

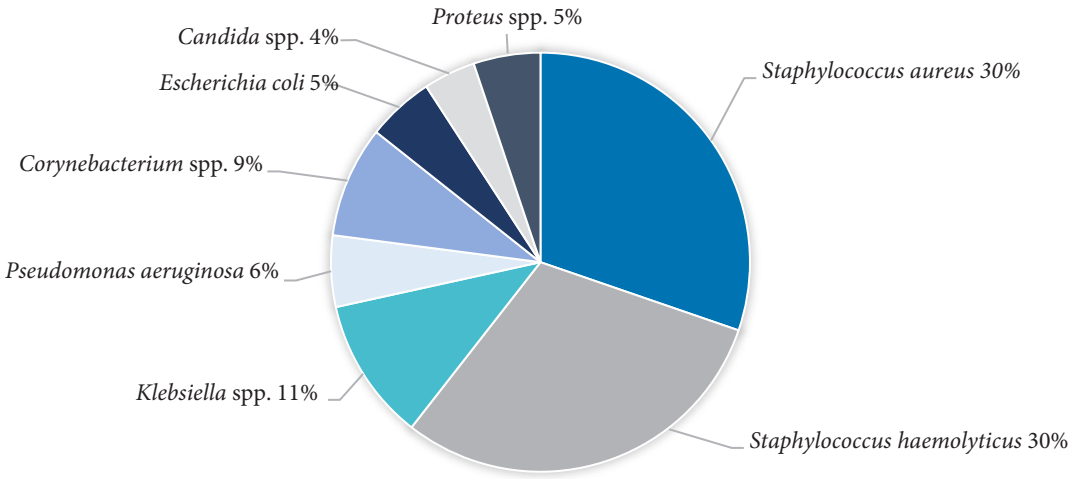

Figure 1. Species diversity of wound content obtained from patients with T2DM with purulent-necrotic complications
Source: compiled by the authors

Research has been conducted not only on the microbial diversity in diabetic foot ulcers, but also on the effectiveness of using specific antibiotics against the identified pathogens. The susceptibility of the isolated bacteria was quite variable. Clinical isolates of *S. aureus* and *S. haemolyticus* showed varying degrees of susceptibility to all studied antibiotics. The most effective antibiotics were tigecycline and vancomycin, used for treating various purulent-septic infections caused by gram-positive bacteria. The susceptibility of these microorganisms to these antibiotics was 89.6% and 100.0%, respectively (see Table 2).

Table 2. Antibiotic susceptibility of microorganisms isolated from purulent-necrotic ulcers in patients with diabetic foot syndrome against the background of type 2 diabetes mellitus

Bacteria / Antibiotic	*Klebsiella* spp.	*S. aureus*	*S. haemolyticus*	*P. aeruginosa*	*Corynebacterium* spp.	*E. coli*	*Proteus* spp.
Ciprofloxacin	R	51.7%	41.4%	100.0%	R	100.0%	R
Ceftazidime	R	37.9%	27.6%	R	R	100.0%	R
Levomycetin	R	62.1%	62.1%	R	50.0%	100.0%	100.0%
Amikacin	27.3%	75.9%	62.1%	100.0%	100.0%	100.0%	100.0%
Azithromycin	R	27.6%	27.6%	R	R	100.0%	100.0%
Ceftriaxone	R	51.7%	41.4%	R	R	R	R
Doxycycline	45.5%	75.9%	62.1%	R	R	R	R
Erythromycin	R	27.6%	34.5%	R	R	R	R
Methicillin	R	51.7%	34.5%	R	R	R	R
Tigecycline	27.3%	89.6%	100.0%	R	100.0%	100.0%	100.0%
Vancomycin	27.3%	89.6%	100.0%	100.0%	100.0%	R	R
Ertapenem	27.3%	51.7%	R	R	R	100.0%	100.0%

Notes: R – resistant
Source: compiled by the authors

In the context of antibiotic therapy, cephalosporins are prescribed, which are chemically and pharmacologically similar to penicillin. The antibiotic ceftriaxone, which is widely used today to treat most bacterial infections and is considered the most successful of the third-generation cephalosporins, showed significantly lower effectiveness: 51.7% for *S. aureus* and 41.4% for *S. haemolyticus*. Similar data were obtained from testing the antibiotic ciprofloxacin. A relatively high susceptibility of *S. aureus* and *S. haemolyticus* (75.9% and 62.1%, respectively) was observed to amikacin, a semisynthetic antibiotic from the third-generation aminoglycosides group, as well as doxycycline, a semisynthetic antibiotic from the broad-spectrum tetracyclines group. The effectiveness of other studied antibiotics ranged from 27.6% to 51.7%. *Klebsiella* spp. was found to be resistant to most antibiotics involved in the study, with only doxycycline showing moderate effectiveness at 45.5% (see Table 2).

It is believed that the presence of *P. aeruginosa* in wound content complicates the purulent-necrotic process

in DFS patients. Bacteriological studies have shown that this pathogen is highly resistant to most studied antibiotics. Only 3 of the 12 antibiotics included in the study were effective: amikacin, vancomycin, and ciprofloxacin, a representative of the second-generation fluoroquinolones. The susceptibility of *P. aeruginosa* to these antibiotics was 100.0% (see Table 2).

Other microorganisms isolated during bacteriological examination showed a varied level of susceptibility to the studied antibiotics. For example, *Corynebacterium* spp., which is usually a commensal species of the skin, was found to be resistant to most antimicrobial agents, with only 4 of them (amikacin, tigecycline, vancomycin, and levomycetin) showing a positive result. The susceptibility to these drugs ranged from 50.0-100.0%. Due to the significant variability in resistance of this pathogen to antimicrobial agents, there is a need for continuous monitoring of the susceptibility of non-diphtheria corynebacteria isolates. The increasing number of studies demonstrates the relevance of researching the resistance of non-diphtherial corynebacteria, as they participate in biofilm formation, which enhances the development of resistance and the recurrence of various infectious diseases. *E. coli* demonstrated a high level of susceptibility (100.0%) to such antimicrobial drugs as ciprofloxacin, ceftazidime, levomycetin, amikacin, azithromycin, tigecycline, and ertapenem (see Table 2).

Among gram-negative microorganisms, *Proteus* spp. is moderately isolated, which belongs to the *Enterobacteriaceae* family. The presence of this pathogen in the biomaterial taken from patients with DM and DFS indicates a serious complication of this infectious process. According to the results of the antibiogram, *Proteus* spp. was resistant to most of the selected antibiotics, with only levomycetin, amikacin, azithromycin, tigecycline, and ertapenem showing high effectiveness (100.0%). However, the results obtained during the study also demonstrated the resistance of *Proteus* spp. to certain antimicrobial drugs (see Table 2). It can be assumed that during the use of antibiotics in the treatment of purulent-necrotic infections, this microorganism acquired resistance to such medicinal products.

Discussion

S. Shahrokh *et al.* [8] as well as A. Atlaw *et al.* [9], in their studies, showed that purulent-necrotic lesions of the foot in type 2 diabetes mellitus are characterised by a significant microbial load. The cause of mild and moderate ulcerations is gram-positive bacteria. At the same time, severe or chronic forms of infection are more often characterised by a polymicrobial nature. The polymicrobial aetiology of DFS was also identified in this study, especially in chronic conditions.

The species variety of microorganisms causing purulent infections is variable and diverse. Quite often, hospital-acquired strains, characterised by a high level of virulence and antibiotic resistance, are observed to be involved. Numerous microbiological studies indicate that the skin in diabetes is characterised by high colonization of such commensal microorganisms as *S. aureus*, *S. epidermidis*, and S. *haemolyticus*. They are the most common agents of purulent ulcers in type 2 diabetes. However, O. Laktionova *et al.* [2], in their studies, showed that representatives of *Pseudomonas* spp. and the *Enterobacteriaceae* family are also quite common. The frequency of detecting these microorganisms ranges from 28.0 to 40.0% [8, 11, 15], which correlates with these findings.

A pressing issue today is the emergence and increase in the number of infections caused by multidrug-resistant bacteria *Klebsiella* spp. This microorganism is known to be the cause of numerous upper respiratory tract infections and is one of the main factors in the development of gastrointestinal diseases. However, in microbial associations, *Klebsiella* spp. is capable of causing purulent-necrotic processes in diabetic foot and is characterised by resistance to antibacterial drugs [15-17]. X. Li *et al.* [16], as well as A.K.P.H. Putra & S. Sundari [17], showed in their studies that this microbe was the cause of necrotic foot lesions in 7.0-11.9% of cases. The same was found by the authors of this study.

Recently, the role of colonizing microorganisms in the development of purulent infections has been increasing, among which *Corynebacterium* spp. plays a leading role [19, 20]. A.N. Khayyat *et al.* [21] identified in their study pathogens with significant tropism for bones and joints, among which *Corynebacterium striatum* is the most common. Some species are associated with urinary tract infections, respiratory infections, surgical wound infections, and endocarditis. However, when *Corynebacterium* spp. are verified in severe infections, including osteomyelitis, they can exhibit pathogenic properties. In such cases, their presence contributes to the development of a pathogenic biofilm. This phenomenon is particularly characteristic of chronic wounds in individuals with diabetes, who have weakened immune responses. Currently, corynebacteria are considered a new pathogen in deep infections of the diabetic foot, with a detection frequency of 4.0-10.0% [11, 18, 21]. The same was found by the authors of this study.

Despite the fact that the majority of skin purulent infections are caused by the presence of staphylococci, the severity of these infections also depends on the presence of other microorganisms, including *Pseudomonas* spp., *E. coli,* and *Proteus* spp. [22]. In conformity with the literature, the following frequencies of these bacteria have been reported: *E. coli* – 17.19% [8]; 7.1% [18]. *Pseudomonas* spp. – 7.54% [8]; 11.9% [18]. *Proteus* spp. – 4.32% [8]; 7.1% [18].

Usually, *P. aeruginosa* is verified quite rarely in diabetic foot ulcers. M.D.M. Bermejo Olano *et al.* [18] as well as W. Sun *et al.* [20] have shown that in patients with DFS on the background of type 2 diabetes, the frequency of *P. aeruginosa* isolation is 7.0 %. The same was found by the authors of this study.This pathogen often causes severe tissue damage in diabetic foot ulcers, leading to sepsis and amputation. The pathogenicity of these bacteria is based on their ability to produce various toxins, proteases, and resist phagocytosis. Clinical isolates of *P. aeruginosa* obtained from chronic wounds are typically resistant to many drugs,

which complicates antibiotic therapy. *Pseudomonas aeruginosa* is considered an opportunistic pathogen known for its metabolic flexibility, exceptional ability to colonise diverse environments, form biofilms, and intrinsic resistance to a wide range of antimicrobial agents due to specific genetic determinants. A.H. Jaber & S.A.F. Almiyah [22] as well as L. Zhuravlyova & O. Keleberda [23] consider that this fact has enabled horizontal gene transfer of resistance genes and allowed P. aeruginosa to overcome the human immune defence.

As the obtained research results and literature data show, numerous purulent-ulcerative lesions of the skin in patients with T2D are usually polymicrobial. Bacteria can exist in the wound as multilayered microbial associations, known as biofilms, surrounded by self-produced extracellular polymeric substances. This structure protects microbial cells from antibacterial agents and the body's immune system, allowing bacteria to proliferate and interfere with wound healing, making such infections difficult to treat.

One of the most important components of comprehensive conservative treatment of a diabetic ulcer is antibacterial therapy, as without correct and timely treatment, patients may develop toxic shock syndrome, leading to multiple organ failure. Today, the resistance of pathogens to antibiotics is considered a significant problem in patients with purulent-necrotic lesions.

According to the recommendations of the Infectious Diseases Society of America (ISDA) regarding antibiotic therapy for moderate and severe cases of diabetic foot syndrome, the main drugs are second and third-generation cephalosporins, combination of β-lactam antibiotics with β-lactamase inhibitors, and carbapenems. Broad-spectrum antibiotics such as ertapenem, ampicillin/sulbactam, imipenem/cilastatin, and piperacillin/tazobactam may also be used. The use of all antibiotics should be based on the bacterial profile of the wound process [19]. Today, there is a decrease in the effectiveness of cephalosporins against staphylococci. For example, S. Shahrokh *et al.* [8] showed resistance of *S. aureus* to ceftriaxone in only 48.0% of cases, which is consistent with these findings.

Klebsiella spp. is characterised by a high degree of resistance. The susceptibility of this pathogen to tigecycline, vancomycin, ertapenem, and amikacin was less than 30.0%, although X. Li *et al.* [16] note a high susceptibility (100.0%) of this microorganism to these antimicrobial agents. The reason for the discrepancy between the obtained results and the literature data is likely the fact that *Klebsiella* spp. is increasingly isolated from purulent lesions in patients with type 2 diabetes as a nosocomial species. As known, such species are characterised by multidrug resistance, the level of which increases every year due to the active use of antibiotics.

Another pathogen with a high level of resistance is *P. aeruginosa.* Only some antibiotics show effectiveness against this pathogen. For example, X. Li *et al.* [16] found 100.0% susceptibility of *P. aeruginosa* to amikacin and ciprofloxacin [14]. The obtained results are consistent with the literature data. The high resistance of this pathogen is likely associated with its ability to form biofilms. Therefore, the WHO has included carbapenem-resistant *P. aeruginosa* in the list of bacteria for which there is a critical need to develop new antibiotics for the treatment of infections caused by this pathogen [23].

Among the microorganisms isolated from the wound content of patients with type 2 diabetes, the role of *Corynebacterium* spp. is increasing. This microbe is characterised by a fairly variable susceptibility to antibiotics. For example, W. Sun *et al.* [20] showed high effectiveness (100.0%) of vancomycin and low activity of erythromycin (resistance level was 91.0-100.0%), ciprofloxacin – 93.3%, doxycycline and tigecycline – 77.8% [24], which is consistent with these findings.

E. coli is susceptible to most antibiotics. According to literature data, antibiotics such as ciprofloxacin, ceftazidime, chloramphenicol, amikacin, azithromycin, tigecycline, and ertapenem are highly effective. For example, the sensitivity of *E. coli* to ertapenem was 96.6 %, and to amikacin – 93.3% [18], to ciprofloxacin – 100.0% [20]. Resistance of *E. coli* to third-generation cephalosporins, specifically to ceftriaxone, has been identified. Such resistance of *E. coli* isolates may be associated with the widespread distribution of beta-lactamases among enterobacteria [8]. The same was found by the authors of this study.

The presence of *Proteus* spp. in purulent-necrotic ulcers, complicates the course of such infection. As known, this microorganism participates in the formation of biofilms, which in turn complicates antibiotic therapy, especially in the case of a diabetic ulcer [19, 23, 24]. Literature data indicate ambiguous and variable susceptibility of this pathogen to antibiotics. For example, A. Atlaw *et al.* [9], as well as X. Li *et al.* [16], noted the high effectiveness of ceftazidime and ceftriaxone (84.6-100.0% and 92.3%, respectively). However, in this study, the authors found resistance of *Proteus* spp. to these antibiotics.

Conclusions

The complex therapy of type 2 diabetes with the complication in the form of purulent-necrotic lesions should be based on microbiological examination of wound content, taking into account the antibiotic susceptibility of the isolated strains. Bacteriological analysis showed that diabetic ulcers usually arise due to the presence of not one, but several types of microorganisms, among which *S. aureus* and *S. haemolyticus* are most commonly encountered (their isolation frequency is 30.0%). The frequency of isolation of other bacteria (*Klebsiella* spp., *Pseudomonas aeruginosa, Corynebacterium* spp., *Escherichia coli, Proteus* spp., and *Candida* spp. fungi), which complement the bacterial diversity of purulent-necrotic ulcers, varies within 4.0-11.0%.

The antibiotic ceftriaxone, which is actively used in the majority of infections caused by microbes, was effective in 51.7% against *S. aureus,* 41.4% against *S. haemolyticus*, and the other identified species showed 100.0% resistance.

The antibiotic amikacin was variably susceptible to all investigated microorganisms: *S. aureus* – 75.9%, *S. haemolyticus* – 62.1%, *Klebsiella* spp. – 27.3%, *Pseudomonas aeruginosa*, *Corynebacterium* spp., *Escherichia coli, Proteus* spp. – 100.0%. In the case of *Klebsiella* spp., it is more advisable to use doxycycline, as compared to other tested drugs, its effectiveness was the highest at 45.5%.

The high effectiveness against most of the isolated strains was observed with tigecycline and vancomycin. The susceptibility of *S. haemolyticus, Pseudomonas aeruginosa,* and *Corynebacterium* spp. to the latter was 100.0%; *S. aureus* was susceptible in 89.6% of cases, but *E. coli* and *Proteus* spp. were resistant. Only *P. aeruginosa* was resistant to tigecycline. Thus, the treatment of type 2 diabetes with complicated foot ulcers should be comprehensive, using rational antibiotic therapy based on microbiological examination. Prospects for further research: to study the rate of development of resistance to modern antibiotics in clinical strains of bacteria that cause purulent inflammatory processes in the DFS.

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None.

Conflict of Interest

The authors declare no conflict of interest.

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Мікробіологічний аналіз вмісту ран у пацієнтів з цукровим діабетом 2 типу з синдромом діабетичної стопи

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Анотація. Важливим компонентом комплексного консервативного лікування діабетичної виразки є антибактеріальна терапія, адже без коректного своєчасного лікування у пацієнтів можливий розвиток синдрому токсичного шоку, що призводить до поліорганної недостатності. Метою дослідження було вивчення видового складу ранового вмісту виразки діабетичної стопи при цукровому діабеті-2 та визначення чутливості виділених мікроорганізмів до антимікробних препаратів. За результатами бактеріологічного дослідження ранового вмісту найчастіше виділяли такі мікроорганізми як: *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Corynebacterium* spp., *Escherichia coli*, *Proteus* spp. та гриби роду *Candida* spp. Проведено дослідження не лише мікробного різноманіття при синдромі діабетичної стопи, а й встановлена ефективність застосування антибактеріального засобу до конкретного виділеного збудника. Найбільш ефективними виявилися антибіотики тігециклін та ванкоміцин, де чутливість вказаних мікроорганізмів становила від 89,6 % до 100 % відповідно. Чутливість *S. aureus* та *S. haemolyticus* до амікацину становила 75,9 % і 62,1 % відповідно. *P. aeruginosa* була чутливою лише до 3 з 12 залучених в дослідження антибіотиків, а саме: амікацин, ванкоміцин та ципрофлоксацин. *Klebsiella* spp. відзначався помірною чутливістю (45,5 %) лише до доксицикліну. *Corynebacterium* spp. виявилися резистентною до більшості досліджуваних антимікробних препаратів і лише до амікацину, тігецикліну, ванкоміцину та левоміцетину рівень чутливості був в межах 50-100 %. *E. coli* продемонструвала високий рівень чутливості (100 %) до таких антимікробних препаратів, як: ципрофлоксацину, цефтазидину, левоміцетину, амікацину, азитроміцину, тігецикліну та ертапенему. *Proteus* spp. були чутливим (100 %) до левоміцетину, амікацину, азитроміцину, тігецикліну та ертапенему. Встановлення мікробного різноманіття ранового вмісту діабетичної виразки та широка антибітикограма дозволить оптимізувати антибіотикотерапію відповідно до протоколу лікування даної патології та запобігти розвитку антибіотикорезистентності, адже саме мікробіом рани визначає розвиток, протікання та ступінь ускладнення гнійно-некротичного процесу

Ключові слова: метаболічне захворювання; інфекція діабетичної стопи; мікроорганізми; антибіотики; антибіотикорезистентність

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Evaluation of a novel osmotically volumetric urine index as a rapid and inexpensive marker for certain renal conditions

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Abstract. An open question remains the assessment of the patient's condition in various kidney diseases using inexpensive laboratory methods. The aim of this study was to evaluate the diagnostic tool, the osmolal-volume index of urine, calculated based on urine density and hourly diuresis. A retrospective study of medical records of 86 intensive care unit patients was carried out (34 – with diabetes insipidus, 30 – with acute renal failure, 22 – with chronic renal failure), as well as a prospective study involving 22 healthy individuals without renal pathology. Urine samples were collected three times over a three-hour period; the index and volume of each fraction were measured and averaged. One-way Analysis of Variance was used to evaluate the influence of study groups on osmotically volumetric urine index; means were separated using Fisher's Least Significant Difference procedure ($p < 0.01$). There was a significant difference between study groups regarding the proposed index ($p < 0.01$), and in healthy individuals it ranged from 8.0 to 12.0. In diabetes insipidus, the proposed index sharply decreased, acquiring values below 1.0. At the initial stage of acute renal failure, its value increased (22.0 ± 5.5), while at the stage of polyuria decreased to 2.0. Chronic renal failure was manifested by the index decrease (4.2 ± 2.1). The osmotically volumetric urine index is a dynamic indicator of the efficiency of excretory and concentration renal function applicable for the field hospitals where necessary lab equipment and reagents are unavailable and history of patient's water consumption, retention, and loss, is known. In case of impaired renal function, this index can vary significantly from 0.02 to 30. The simplicity of the method, its non-invasiveness, plus as the communicativeness, deserve the introduction of this marker into clinical practice

Keywords: renal failure; diabetes insipidus; urine density; diuresis rate; osmotically volumetric urine index

Introduction

A wide variety of reasons, namely, trauma, surgery, acute and chronic inflammatory diseases, etc., can lead to body homeostasis disorders in patients which require intensive care. The kidneys are one of the main organs for homeostasis regulation due to the processes of filtration, reabsorption, secretion, with final urine release [1]. Kidneys

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regulate blood volume, osmotic blood pressure (osmoregulation), and concentration of blood organic substances and low-molecular weight electrolytes (ion regulation) [1]. Therefore, early and timely diagnosis of impaired renal function often plays a crucial role in understanding the mechanism of pathophysiological processes occurring in the body, evaluating the effectiveness of intensive care, and preventing possible further disorders of homeostasis.

O. Hnativ & M. Korda [2] studied and thoughtfully reviewed the modelling of hyperosmolar hypohydration in rats, manifested by diabetes insipidus (DI), and its influence on overall water-salt balance, homeostasis, and functional disorders. Patients with kidney pathology in intensive care units frequently require lab tests to check kidney functionality, which may be impossible in the case of field hospitals, where numerous patients must be attended simultaneously due to natural or man-made disasters, civil unrest, or wars. Under normal conditions, standard lab chemical tests include blood analysis (serum creatinine, blood urea nitrogen, cystatin C levels), as well as urinalysis (urine protein, albumin, creatinine levels, blood cells, glucose, microbiological analysis, among others) [3, 4]. Scientists R. Jin *et al.* [5] and R. Hojs *et al.* [6] suggested several derived indices, such as kidney estimated glomerular filtration rate (eGFR) as a function of serum creatinine, age, gender, race, body surface area, serum cystatin C; albumin to creatinine ratio (ACR) [7], which provides more in-depth evaluation of renal status. Structural changes in kidney tissue can be observed with either a computed tomography (CT) scan or ultrasound. In case of the absence of sophisticated equipment and chemical reagents, rapid and fairly useful information can be obtained with physical methods of examination, such as urine appearance, its hourly and daily volume, gravity measured with either manual/digital refractometer, hydrometer, or by test strips, as well as urine osmolality measured by urine freezing point depression [8-10].

In the case of limited financial, human, and other resources, when numerous patients are admitted simultaneously to low-equipped hospitals in urgent situations, the following criteria may become the most important in the diagnosis of the impaired renal function: non-invasiveness, simplicity, speed, and informativeness of the results. The aim of the current study was to evaluate a rapid aggregative diagnostic indicator based on hourly urine volume and its gravity, using a retrospective approach with the data from the groups of patients with certain pathologies and a control group, which included healthy individuals.

Materials and Methods

A retrospective study of medical records was carried out, which included total 86 intensive care unit (ICU) patients (34 – with DI group; 30 – with acute renal failure (ARF) group; 22 – with chronic renal failure (CRF) group) and 22 healthy individuals without renal pathology, control (C) group. The study was conducted on the premises of Ternopil University Hospital and clinical hospital "Feofania" of the State Affairs Department using records dated from 2016 till 2021. 22 healthy individuals without renal pathology (C group) were randomly selected from resident doctors of Ternopil University Hospital to match gender ratio of ICU patients as an ongoing part of the experiment.

Exclusion criteria for the study included age over 60 years, history of recent diuretics use, benign prostatic hyperplasia, type I and type II diabetes mellitus. Average age ± standard deviation and male/female ratio for the DI group, ARF group, CRF group, and C group, were 43 ± 5 years old and 1.2, 40 ± 5 years old and 1.1, 39 ± 6 years old and 1.2, 27±3 years old and 1.2, respectively. Individuals aged over two sigmas below or above the average age value were excluded from the study. Diabetes insipidus was diagnosed according to Robertson [11], with criteria including hypotonic (<300 $mOsm*kg^{-1}$) urine output above 50 $mL*day^{-1}*kg^{-1}$ body weight and polydipsia (>3.0 $L*day^{-1}$). Acute renal failure and chronic renal failure were diagnosed according to KDIGO guidelines [3, 4]. Using standard clinical and biochemical examinations at the Department of Anaesthesiology and Intensive Care, ICU patients were catheterised with urinary catheters and were subjected to the targeted study of renal excretory and concentrating ability.

The diuresis rate was measured as a urine volume excreted through the kidneys per one hour. Continuous checking of the diuresis rate in ICU patients which have urinary catheters is a routine procedure performed by medical personnel but supervised by an anaesthesiologist on duty. Three consecutive urine volumes over three one-hour periods were collected hourly and analysed after presumptive onset of kidney malfunctioning symptoms before diuretics use with standard physiological water and nutrient supplementation. Healthy individuals (C group) were advised to consume daily-recommended drinking water volume (2.0 litres per day) and to avoid using any type of diuretic drugs and caffeine. The urine samples from healthy individuals were collected after noon over three consecutive hours, and hourly urine volume was recorded. Each portion of the urine excreted per hour was analysed for its gravity ($g*L^{-1}$) using a refractometer (Fisherbrand™ Handheld Analogue Clinical Refractometer, Thermo Fisher Scientific, Waltham, MA, US). The results from each experimental subject (three values) were averaged and used for further data analysis and interpretation.

A method for assessing renal function based on the simultaneous measurement of urine density and diuresis rate was previously suggested and named "osmotically volumetric urine index" (OVUI, $g*h*mL^{-1}*L^{-1}$) by previous research [12]. The OVUI calculation formula is as follows:

$$OVUI = (UG - 1000) * 100/(3 * Vhour), \quad (1)$$

where *OVUI* is osmotically volumetric urine index, *UG* – urine gravity, measured by refractometer, $g*L^{-1}$, *Vhour* – diuresis rate per hour (urine volume in mL, excreted per hour).

The above-mentioned index, OVUI, was evaluated, and a range of its values were established for healthy

individuals and patients with renal pathology, as well as with diabetes insipidus. It is important to note that refractometric examination of the patient's urine can be performed directly near the patient's bedside, with minimal time and biological fluid used. This allows monitoring of renal function every hour, assessing the dynamics of the pathological process and intensive care efficiency.

Assuming a normal distribution of the calculated OVUI value, one-way ANOVA was used to analyse the influence of the study group (group C, group DI, group CRF, group ARF) on the OVUI value. If the influence of the study groups was significant ($p < 0.01$), means were separated using the Fisher LSD method. Statistical analysis was performed using commercially available software Statistica ver. 10.0 (StatSoft Inc, Tulsa, OK, US).

Standard written consent on use and processing of personal data was obtained from each individual involved in the study. The study was performed in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving human subjects [13] and approved by the local Institutional Research Ethics Committee of I. Horbachevsky Ternopil National Medical University Ministry of Health of Ukraine.

Results and Discussion

It has been established that the osmotically volumetric urine index may be considered as an effective marker of impaired renal function. The results of analysis of groups DI, ARF, CRF, together with the data from group C, are shown in Table 1.

Table 1. OVUI of the investigated groups

Group	Ave OVUI ± st. dev., $g*h*mL^{-1}*L^{-1}$	
C	10.0 ± 1.2	*b
DI	0.40 ± 0.13	d
ARF, early phase	22.0 ± 5.5	a
CRF	4.2 ± 2.1	c

Notes: * – means with the same letters are not significantly different ($p<0.01$)
Source: compiled by the authors

Healthy individuals without renal pathology. There was a significant influence of study groups on the calculated OVUI value ($p < 0.01$). Healthy individuals (group C) who received food and water consumption in the amount of physiological needs, had an average OVUI ± standard deviation of 10±1.2, ranging from 8.0 to 12.0.

The OVUI decreased below 8.0 with an excessive water consumption in healthy individuals, while it increased above 12.0 when water intake into the body was limited (data not shown). Similarly, when conducting intensive care in patients with preserved renal function, the growth of OVUI above 12.0 indicated insufficient volemic support or unresolved water deficiency in the body, while excessive hemodilution, as well as the use of forced diuresis, was manifested by a decrease in OVUI below 8.0 (data not shown). The correlation between the diuresis rate and the urine gravity in healthy individuals in normal physiological conditions and at different options of the body hydration are shown in Figure 1.

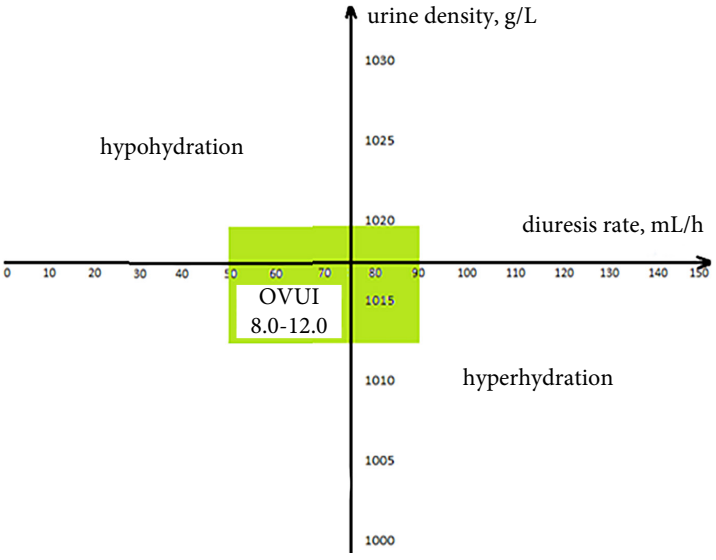

Figure 1. Osmotically volumetric urine index in healthy individuals under physiological fluid supplementation, retention, and loss (green box)
Source: compiled by the authors

In diabetes insipidus (central and nephrogenic), OVUI ranged from 0.2 to 0.6, and correlated with severity of the pathology, with lower values detected in more severe cases (Fig. 2). Based on previous experience with corrective actions, the efficiency of homeostasis correction in diabetes insipidus is evidenced by the dynamics of the constant growth of the OVUI above 1.0 with it eventually reaching normal physiological values. In the case presented here, diabetes insipidus was manifested by the OVUI shift to the right and down (Fig. 2).

Acute renal failure. At a shock stage of acute renal failure, OVUI increased above 15.0 up to 30.0 $g*h*mL^{-1}*L^{-1}$ (Fig. 3).

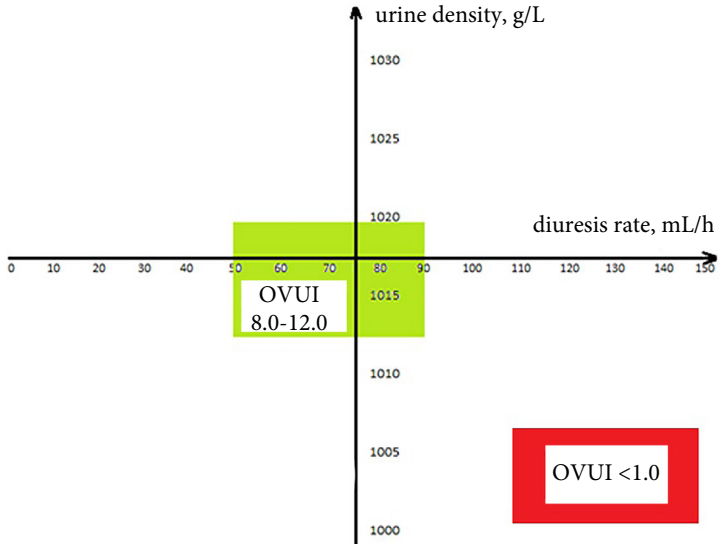

Figure 2. Osmotically volumetric urine index in diabetes insipidus (red box). OVUI shifts to the right and down compared to the norm

Source: compiled by the authors

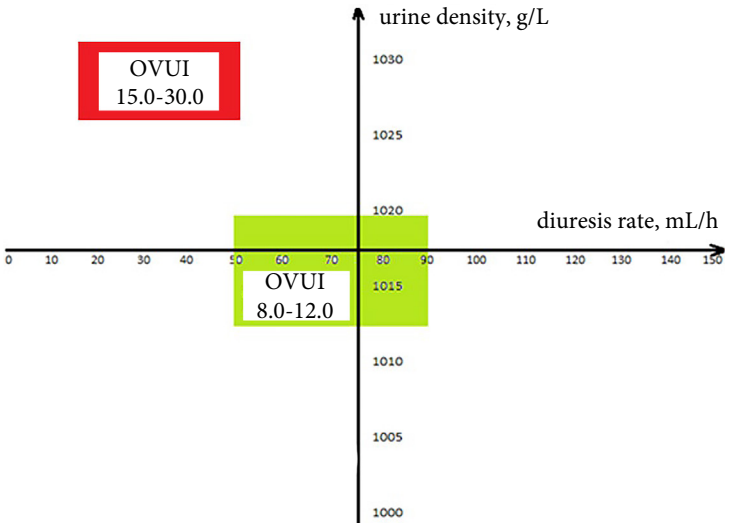

Figure 3. Osmotically volumetric urine index in acute renal failure, early phase (red box). OVUI shifts to the left and up compared to the norm

Source: compiled by the authors

In acute renal failure in the polyuria phase, the OVUI falls below normal, approaching 1.0. With the administration of particular drugs (X-ray contrast agents, mannitol, dextrans, nephrotoxic antibiotics) OVUI increases above 12.0 due to the increase in the urine density. OVUI normalization indicates the restoration of kidneys excretory and concentrating capacity and the patient's recovery. On the diagram, it is manifested by the OVUI shift back to the right and down.

Chronic renal failure was manifested by a decrease in osmotically volumetric urine index below 8.0 and to 2.0-3.0. There is a direct correlation between the OVUI and the

disease severity: the lower OVUI values indicate that tubular function is largely impaired, and glomerular function is still sufficient (Fig. 4). To summarise, the shown data suggested that OVUI in its presented form may be a valuable tool for evaluation of such conditions as diabetes insipidus, acute and chronic renal failure as compared to healthy individuals.

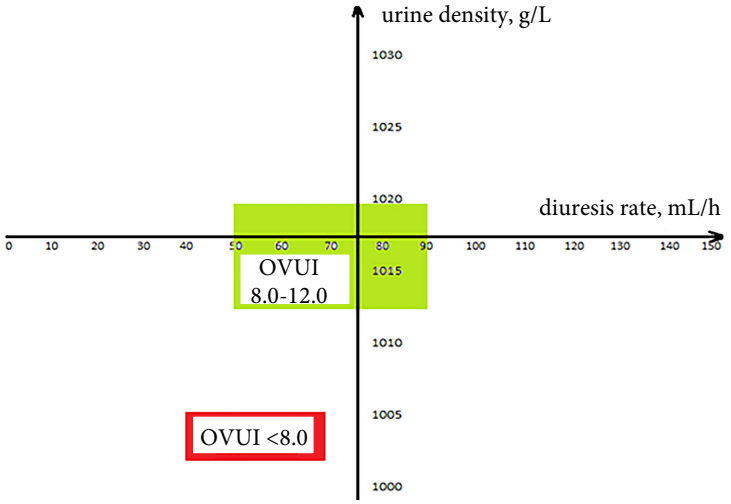

Figure 4. Osmotically volumetric urine index in chronic renal failure (red box). OVUI shifts to the left and bottom compared to the norm

Source: compiled by the authors

A healthy person under normal physiological conditions excretes urine at a rate of 0.8-1.0 mL*kg^{-1} body weight within one hour, with urine gravity ranging from 1,012 to 1,025 g*L^{-1}, which indicates the ability of the kidneys to filter and concentrate biological fluid [1]. Moreover, in acute kidney injury, one of the sufficient signs is urine volume of below 0.5 mL*kg^{-1}*h^{-1} for consecutive 6 hours [3]. Although urine flow rate alone is a poor measurement of kidney function, oliguria generally reflects a decreased GFR. However, according to KDIGO [3, 4], acute kidney disease or chronic kidney disease may take progress even without any substantial decrease in urine flow rate. According to M. Pradella *et al.* [8], Similarly, urine gravity can physiologically fall below 1,012 in the case of high fluid intake or rise above 1,025 after taking no fluids for 12 hours overnight.

Hypersthenuria, or increased concentration of solutes in urine, causes an increase in urine gravity and may be associated with dehydration, emesis, diarrhoea, excessive sweating, urinary tract infection, glycosuria, hepatorenal syndrome, renal artery stenosis, decreased kidney blood flow as a result of heart failure. Urine gravity greater than 1,035 g*L^{-1} is consistent with obvious dehydration [14]. The decreased concentration of solutes in urine, or hyposthenuria, may be associated with pyelonephritis, renal failure, diabetes insipidus, interstitial nephritis, acute tubular necrosis, and excessive fluid intake.

The solute concentration in urine can be measured either by specific gravity (hydrometer, refractometer, test strips), which depends on the number and weight of the solute particles in urine, or by osmolality (urine freezing point depression method), which depends only on the number of solute particles [8, 15]. As hydrometer use requires larger urine volumes, which is inconvenient and impossible in the case of oliguria, refractometers and test strips become a viable option. Though studies, S.J. Barton & S.S. Holmes [16] showed promising results for urine specific gravity (USG) stick test strips, later studies questioned their usefulness, mentioning high urine pH (above 7.0), urine glucose and urine protein as interfering factors, as well as overall lack of correlation [15, 17, 18], which leaves refractometers as the most viable option for USG and UG measurements.

The OVUI value (Fig. 1) for healthy individuals should be taken with certain caution, as D.J. Casa *et al.* [19] classified well-hydrated individuals with UG at below 1010, minimally dehydrated – with UG between 1,010 and 1,020, and significantly dehydrated – with UG between 1,021 and 1,030, which do not fall within OVUI 8.0-12.0 box.

During diabetes insipidus (central and nephrogenic), previous study also showed that OVUI was one of the earliest, most informative and efficient diagnostic criteria, especially starting from the first hours of pathology development [12]. Similar to the presented here results, this index also decreased sharply, gaining values even below 0.5 ($p < 0.001$) [12]. It was also noticed that typically such OVUI shifts in diabetes insipidus occurred long before the development of clinical signs of impaired hydration of the body, hypernatraemia and hyperosmolarity of blood plasma inherent in patients with such condition [12, 20].

Continuous OVUI measurements and analysis make it possible to determine at what level (glomerular or tubular) renal failure occurred. Usually, diuresis decrease to 0.5 mL*kg^{-1}*h^{-1} ascertains oligoanuria and is a serious sign of acute renal failure [3, 4]. As expected, an increase in the index was also characteristic of parenchymal acute renal

injury, such as the initial stage of acute diffuse glomerulonephritis and hepatorenal syndrome. As diuresis is restored, this indicator decreases, approaching the norm. However, a decrease in OVUI against the background of a low rate of diuresis, indicates the exaltation of the kidneys concentrating capacity – i.e., the involvement of the renal tubules in the pathological process. In the polyuria stage acute renal failure, the OVUI fell below normal, approaching 1.0.

During chronic renal failure, low OVUI values (<5.0), which do not change during the day when examining sequential portions of urine, indicate a pronounced renal concentration failure, when the density of glomerular filtrate equals to the density of final excreted urine and serve as a marker of an unfavourable course of diffuse chronic glomerulonephritis and pyelonephritis. In the terminal stage of nephrosclerosis due to the progression of oliguria against the background of low urine density, the osmotically volumetric urine index may again acquire values close to normal ones.

There are some drawbacks in using refractometers for urine solute concentration measurements. For example, osmolality measurement as an alternative to refractometry is considered a golden standard and is normally used for more detailed analysis in ARF and CRF, but is less convenient [17, 21]. However, some researchers even proposed simple equations to derive osmolality values from refractometer-read UG [22, 23]. Contrary, C.E. Costa *et al.* [17] stated that refractometry-obtained UG could not substitute more precise and informative osmolality value. The authors wrote that the correlation coefficient between osmolality and refractometer-read UG in many cases was weak even after adjustments with urine glucose and protein presence, and only osmolality had a significant non-linear correlation with serum creatinine [15].

To summarise, though both investigated urine parameters (urine hourly volume and refractometer-read UG) may vary significantly even in healthy individuals, their composite-derived index can be useful in clinical settings, especially if history of patient's fluid replacement or loss, as well as previous medicine administration and general anamnesis, are well-known. However, for final diagnosis and treatment options, serum/urine creatinine levels, urine albumin levels, serum cystatin C levels, urine output, and composite eGFR and ACR values should be used instead.

Conclusions

As the main goal of the current research was evaluation of a novel osmotically volumetric urine index as a rapid and inexpensive marker for certain renal conditions, the targeted aims were achieved. Osmotically volumetric urine index may be used as a dynamic indicator of the efficiency of excretory and concentration functions of the kidneys in case of emergency, lack of appropriate equipment, and known anamnesis and history of drug administration, fluid replacement and loss. It was shown that under normal physiological conditions, OVUI ranged between of 8.0-12.0, but could vary widely even in healthy individuals. However, in the cases of impaired renal function, this index varied significantly from 0.02 to 30. Based on continuous OVUI monitoring, it may be possible to predict renal pathology, as well as track its progress and the adequacy of intensive care. The simplicity of the method, its communicativeness and non-invasiveness, make OVUI measurements a useful tool at the point of introduction of the marker into the clinical settings. Further research is warranted to explore OVUI's utility in diagnosing specific kidney diseases, its correlation with established markers, and its role in predicting patient outcomes. Additionally, comparing OVUI with existing methods and investigating its applications in various clinical settings will be crucial. Ultimately, OVUI holds promise for improving early detection and management of kidney dysfunction, leading to better patient care and potentially reducing healthcare costs.

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Conflict of Interest

The authors declare no conflict of interest.

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Оцінка нового осмотично-об'ємного індексу сечі як швидкого та недорогого маркеру для певних захворювань нирок

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Анотація. Відкритим питанням залишається оцінка стану пацієнта при різноманітних захворюваннях нирок, використовуючи недорогі лабораторні методи. Метою цього дослідження була оцінка діагностичного інструменту, осмотично-об'ємного індексу сечі, що розраховується на основі щільності сечі і щогодинного діурезу. Проведено ретроспективне дослідження медичної документації 86 пацієнтів відділення інтенсивної терапії (34 – з нецукровим діабетом, 30 – з гострою нирковою недостатністю, 22 – з хронічною нирковою недостатністю), а також проспективне дослідження за участю 22 здорових осіб без патології нирок. Зразки сечі відбирали тричі протягом трьох послідовних годин; значення запропонованого індексу та об'єм кожної фракції вимірювали та усереднювали. Однофакторний дисперсійний аналіз використовувався для оцінки впливу досліджуваних груп пацієнтів на показник запропонованого індексу, статистична значимість різниці між величинами була оцінена критерієм Фішера ($p < 0,01$). Між досліджуваними групами спостерігалась статистично достовірна різниця щодо запропонованого індексу ($p<0,01$), в той час як у здорових осіб вона коливалася від 8,0 до 12,0. При нецукровому діабеті індекс різко знижувався, набуваючи значень нижче 1,0. На початковій стадії гострої ниркової недостатності індекс зростав ($22,0 \pm 5,5$), а на стадії поліурії знижувався до 2,0. Хронічна ниркова недостатність характеризувалася зниженням запропонованого показника ($4,2 \pm 2,1$). Отже, осмотично-об'ємний індекс сечі – це динамічний індикатор ефективності виділення та концентрації ниркової функції, який може застосовуватися у польових госпіталях, де немає необхідного лабораторного обладнання та реагентів та відома історія споживання, затримки та втрати води пацієнтом. При порушенні функції нирок цей показник може істотно коливатися від 0,02 до 30. Простота методу, його неінвазивність, а також інформативність заслуговують на впровадження цього маркера в клінічну практику

Ключові слова: ниркова недостатність; нецукровий діабет; щільність сечі; швидкість діурезу; осмотично-об'ємний індекс сечі

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Anthropometric indices, a predictive marker for stroke and other metabolic disorders

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Abstract. Although a lot of studies have been conducted on stroke and its prevention, stroke is yet a common occurrence in Nigeria. Hence, it becomes imperative to identify reliable and easily accessible predictive markers for stroke and other metabolic disorders. This research therefore aimed to investigate the prediction of stroke and other metabolic disorders using anthropometric indices. The study population included 211 subjects with 86 males and 125 females at an age range of 26 to 95 years, and the selection process employed a simple random sampling method. Anthropometric indices like body mass index, waist-hip ratio, waist circumference, hip circumference and abdominal circumference were measured. The body mass index and waist-hip ratio in females compared to their male counterparts were 32.32 and 28.90 respectively for body mass index and 0.95 and 0.94 respectively for the waist-hip ratio. the male subjects had an abnormal body mass index, with peak values at ages greater than 36. The most number of stroke and metabolic disease patients were documented at the ages of 56 to 65 years for both male and female subjects. All females diagnosed with stroke and other metabolic diseases had an abnormal waist-hip ratio (>0.80) while most of the males had a high normal waist-hip ratio. There was also a statistically significant sex variation ($p \leq 0.05$) in the body mass index and hip circumference for patients diagnosed with stroke and other metabolic diseases. No correlation exists between age and the occurrence of stroke and other metabolic diseases. The findings show that the anthropometric indices: body mass index, waist-hip ratio and hip circumference were better predictive markers than the age of the subjects. Again, the study showed that high normal waist-hip ratio was a good predictive marker for stroke and other metabolic disorders in males. The findings of this study will be relevant for medical experts, nutritionists, and stroke prevention associations

Keywords: anthropometry; body mass index; cardiovascular disease; hip circumference; waist-hip ratio

Introduction

Stroke is a cerebrovascular disease resulting from a haemorrhage of a blood vessel in the brain and arising from several traumatic and metabolic conditions. R. Lalo *et al.* [1] in his study documented that over 65% of stroke

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patients had diabetes and over 47% had hypertension. Most studies conducted have linked stroke to cardiac origin, with cardioembolism being the major precipitating condition and resulting in an ischaemic stroke [2, 3]. Therefore, considering the unreported cases and as well those not accounted for at all, makes stroke an enormous socio-medical burden. Several risk factors have however been documented for stroke and among all, obesity being highly implicated [4] but with no much precision to which anthropometric parameter is of a greater risk. Despite advancements in stroke research and prevention, there is a need to identify reliable and easily accessible predictive markers for stroke and other metabolic diseases. It is being documented from current research that hypertension, sinus arteriolosclerosis and as well venous thrombosis in the young are the primary contributors to stroke [5], and these conditions themselves are primarily influenced by anthropometric factors.

Waist circumference (WC) serves as a valuable metric for measuring abdominal fat accumulation. A recent study conducted on adults of Shanghai in China on the association between Body mass index (BMI) and Stroke documented a U-Shaped relationship with attenuations in both stroke and mortality in much advanced age groups [6]. D.H. Kim *et al.* [7] discovered positive associations between WC variations and stroke occurrence and ultimately ending in death.

In a population-based study conducted in southwestern Nigeria, E. Taiwo & L.O.O. Thanni [8] stated that WC emerged as a reliable predictor of cardiovascular risk factors like hypertension and diabetes. It was documented that the association between hip circumference (HC) and stroke risk differs slightly in individuals based on age and height. It was also demonstrated by Y. Li *et al.* [9], that both the HC and the WC have independent and opposite effects on stroke risk, with waist-hip ratio (WHR) having a more significant impact. Therefore, although HC be related to stroke risk, this relationship is often strongly described in terms of the WHR [10]. In the assessment of central obesity, WC and WHR have become profoundly common parameters, and these indices have been related to an increase in cardiovascular diseases [11]. It is evident following the findings from previous studies that anthropometric indices, especially abnormal BMI and WHR, are implicated in cardiovascular diseases. Obesity and high waist circumferences have been tagged as high-risk factors in the occurrence of diabetes and hypertension. These as well are high risk factors in stroke occurrence. This research therefore aimed to investigate the prediction of stroke occurrence as well as other metabolic diseases using the measurement of some anthropometric parameters.

Materials and Methods

This is both a retrospective and prospective cross-sectional study. To align with the research objectives, the selection of the study population encompasses individuals who have received a diagnosis of stroke at the neurologic clinic of the Rivers State University Teaching Hospital (RSUTH) and other patients who have been diagnosed with hypertension, diabetes and other heart and metabolic related disease. Diagnosis of these health conditions was confirmed from patient's questionnaire and hospital folders collected over a period of three years from 2020 to 2023. Stroke cases due to viral illnesses and trauma were excluded from this study. A sample size of 211 participants with 86 males and 125 females at an age range of 26 to 95 years were recruited, and the selection process employed a simple random sampling method.

Data were collected from participants using a questionnaire with information covering demographic characteristics like gender, age, medical conditions such as stroke and stroke risk factors such as hypertension, diabetes, and other heart related diseases. Participants were interviewed once, at a single time point, providing a snapshot of the relationship between exposures and outcomes at that moment. Patients were recruited to fill the study questions after an informed consent. Patients who had cognitive disabilities were used after relatives had signed the questionnaire. Individuals who excluded details were removed.

Anthropometric indices measurement and calculation. Weight was measured with subjects minimally clothed without shoes, using digital scales and recorded to the nearest 0.01 kg. Height was measured in a standing position without shoes, using a tape meter with shoulders in a normal alignment. BMI was calculated as weight (*kg*) divided by square of height (m^2).

$$\text{BMI} = \frac{Weight\ (kg)}{Height\ (m^2)}, \tag{1}$$

where BMI – body mass index.

WC was measured at umbilical level, using an unstretched tape meter to wrap around the body level between the jugular notch superiorly and pubic symphysis inferiorly, without any pressure to body surface and HC at around the widest part of one's hips, typically just below the greater trochanter and above the pubic symphysis. The measuring tape was made to run parallel to the ground and fits comfortably without being excessively tight in both measurements.

WC was measured using an unstretched tape meter, with the measuring tape placed horizontally around the widest part of the person's hips and buttocks. WHR was calculated as WC *(cm)* divided by HC *(cm)*.

$$\text{WHR} = \frac{WC}{HC}, \tag{2}$$

where WHR – waist-hip-ratio, *WC* – waist circumference, *HC* – hip circumference.

Data was analysed using SPSS version 23 and Microsoft Excel. Statistical tools such as mean and standard deviation as well as the student t test, ANOVA and Pearson's correlation were utilised for data analysis. A p-value of ≤0.05 was considered statistically significant.

All subjects who participated in the study were given an informed consent as to the relevance of the study and that the measurements portend no potential hazards to

their body and health. They were assured of confidentiality of their data and informed of private access to outcome of research on request. Ethical principles were observed in the work in accordance to the Helsinki Declaration [12]. Ethical approval was granted by the Rivers State University Teaching Hospital Research Ethics Committee (RSUTH/REC/2023350).

Results

The results of the study presented in Tables 1 to 10 provide important information about the population of patients diagnosed with stroke and other metabolic diseases. The tables provide detailed statistics on age, sex and anthropometric characteristics of the groups studied. The research shows the relationship between the occurrence of stroke and other metabolic diseases with anthropometry. This could inform researchers and clinicians interventional measures to adopt when managing patients and as well preventive care. Table 1 shows greater BMI and WHR in females compared to their male counterparts at values of 32.32 and 28.90 respectively for BMI and 0.95 and 0.94 respectively for the WHR.

Table 1. Descriptive statistics of subjects diagnosed with stroke and other metabolic diseases

	Sex	N	Mean	Std. Deviation	Std. Error
Age (years.)	Male	86	60.03	11.23	1.21
	Female	125	57.48	13.09	1.17
BMI (kg/m²)	Male	86	28.90	5.26	0.57
	Female	125	32.32	8.08	0.72
WHR	Male	86	0.94	0.06	0.01
	Female	125	0.95	0.21	0.02
WC (cm)	Male	86	94.72	13.69	1.48
	Female	125	97.56	12.45	1.11
HC (cm)	Male	86	100.71	12.52	1.35
	Female	125	105.08	14.06	1.26
AC (cm)	Male	86	94.72	13.69	1.48
	Female	125	97.56	12.45	1.11

Notes: BMI – body mass index; WHR – waist-hip ratio; WC – waist circumference; HC – hip circumference; AC – abdominal circumference; N – number of participants
Source: compiled by the authors

Both male and female subjects showed slight variations in their anthropometric indices, with females having higher number of abnormal WHR and BMI. This could also explain the reason of having more female than male patients. Table 2 shows that most male subjects had an abnormal BMI, with peak values at ages greater than 36. The greatest number of patients were documented at the ages of 56 to 65 years.

Table 2. BMI of subjects diagnosed with stroke and other metabolic diseases

Age groups (years)	N		Mean height(m²)		Mean weight (kg)		BMI (kg/m²)	
	M	F	M	F	M	F	M	F
16-25	0	1	0	2.62	0	78.00	0	29.70
26-35	2	5	3.12	2.78	75.00	76.10	23.93	27.90
36-45	6	18	2.78	2.36	81.30	70.80	30.40	30.40
46-55	18	24	2.51	2.42	76.00	77.90	30.43	32.60
56-65	38	44	2.57	2.29	70.60	73.60	27.70	33.00
66-75	14	24	2.41	2.18	71.70	71.30	30.20	33.60
76-85	7	6	2.59	2.28	74.40	62.90	30.50	28.70
86-95	1	2	2.34	1.94	58.00	59.50	24.80	31.10

Notes: N – number of subjects; BMI – body mass index; N – number of subjects; M – male; F– female
Source: compiled by the authors

Normal BMI values in the range of 18.5 to 24.9 are considered normal by World Health Organization (WHO) standards at the time of update in 2024 [13]. Most male patients fell into the middle age class, and this appears to be the period with much weight gain and fat accumulation. The table also shows that all female subjects had abnormal BMI (BMI > 24.9 is abnormal according to WHO) with a greater number of patients within the age range of 56 and 65 years. Stroke cases appear commoner in females of the middle age class. Table 3 shows most male subjects with high normal WHR. A greater number of patients fell into the age range of 46 and 75, with the highest numbers at the ages of 56 to 65 years.

Table 3. WHR of subjects diagnosed with stroke and other metabolic diseases

Age groups (years)	N		Mean WC (CM)		Mean HC (CM)		WHR	
	M	F	M	F	M	F	M	F
16-25	0	1	0	90	0	108	0	0.83
26-35	2	5	83.5	91.5	98	106.3	0.85	0.87
36-45	6	18	93.8	91.9	100.7	102.2	0.93	0.90
46-55	18	24	97.4	97.4	103.1	102.9	0.94	1.01
56-65	38	44	95.1	98.9	101.3	105.9	0.94	0.94
66-75	14	24	88.9	101.2	93.7	106.9	0.94	0.96
76-85	7	6	99.1	100.8	106.3	103.7	0.93	0.98
86-95	1	2	107	102.5	101	102	1.10	1.01

Notes: WHR – waist-hip ratio; WC – waist circumference; HC – hip circumference; N – number of subjects; M – male; F –female
Source: compiled by the authors

Normal values for WHR, males = <0.95 and females = <0.80 are considered normal by WHO standards at the time of update in 2024. [13]. The table also shows that all females diagnosed of stroke and other metabolic diseases had an abnormal WHR (WHR > 0.80 in females are considered abnormal according to WHO). Most patients fell into the category with very high WHR. Abnormal WHR appears commoner among female subjects as compared to their male counterparts. Table 4 shows statistically significant sex variation ($p \leq 0.05$) in the BMI and HC for patients diagnosed with stroke and other metabolic diseases.

Table 4. T-test to determine sex variation among subjects diagnosed with stroke and other metabolic diseases

Anthropometric indices		T value	t-critical	Df	Sig. (2-tailed)	Mean difference	Std. error difference
Age (years)	Equal variances assumed	1.48	0.68	209.00	0.14	2.56	1.73
	Equal variances not assumed	1.52	0.68	198.98	0.13	2.56	1.68
BMI (kg/m²)	Equal variances assumed	-3.45	0.68	209.00	0.00	-3.42	0.99
	Equal variances not assumed	-3.72	0.68	208.42	0.00*	-3.42	0.92
WHR	Equal variances assumed	-0.21	0.68	209.00	0.84	-0.00	0.02
	Equal variances not assumed	-0.25	0.68	153.73	0.81	-0.00	0.02
WC	Equal variances assumed	-1.56	0.68	209.00	0.12	-2.84	1.82
	Equal variances not assumed	-1.54	0.68	171.22	0.13	-2.84	1.85
HC	Equal variances assumed	-2.32	0.68	209.00	0.02*	-4.37	1.89
	Equal variances not assumed	-2.37	0.68	195.59	0.02	-4.37	1.85
AC	Equal variances assumed	-1.56	0.68	209.00	0.12	-2.84	1.82
	Equal variances not assumed	-1.54	0.68	171.22	0.13	-2.84	1.85

Notes: BMI – body mass index; WHR – waist-hip ratio; WC – waist circumference; HC – hip circumference; AC – abdominal circumference; * – significance ($p \leq 0.05$)
Source: compiled by the authors

The study shows that sex differences occur with the BMI and HC, and this could suggest the reason for having more female cases of stroke, as shown in earlier tables. Table 5 shows no statistically significant difference in the various anthropometric indices studied across the male age groups.

Table 5. Variation among different age groups of male subjects diagnosed with stroke and other metabolic diseases using ANOVA

Anthropometric indices		Sum of squares	df	Mean square	F value	F-critical	P value
BMI (kg/m²)	Between groups	115.63	3	38.54	1.414	2.72	0.25
	Within groups	2,235.22	82	27.26			
	Total	2,350.84	85				
WHR	Between groups	0.02	3	0.01	1.61	2.72	0.19
	Within groups	0.31	82	0.00			
	Total	0.33	85				
WC (cm)	Between groups	646.23	3	215.41	1.16	2.72	0.33
	Within groups	15,286.04	82	186.42			
	Total	15,932.27	85				

Continued Table 5

Anthropometric indices		Sum of squares	df	Mean square	F value	F-critical	P value
HC (cm)	Between groups	398.63	3	132.88	0.84	2.72	0.47
	Within groups	12,915.11	82	157.50			
	Total	13,313.73	85				
AC (cm)	Between groups	646.23	3	215.41	1.16	2.72	0.33
	Within groups	15,286.04	82	186.42			
	Total	15,932.27	85				

Notes: BMI – body mass index; WHR – waist-hip ratio; WC – waist circumference; HC – hip circumference; AC – abdominal circumference; df – degree of freedom
Source: compiled by the authors

All male subjects diagnosed with stroke and other metabolic diseases had similar anthropometric measurements, irrespective of their age differences. Table 6 shows no statistically significant difference in the various anthropometric indices studied across the female age groups.

All female subjects diagnosed with stroke and other metabolic diseases had similar anthropometric measurements, irrespective of their age differences. Table 7 shows a very weak positive correlation between age and the anthropometric indices studied for both male and female subjects.

Table 6. Variation among different age groups of female subjects diagnosed with stroke and other metabolic diseases using ANOVA

Anthropometric indices		Sum of squares	df	Mean square	F value	F-critical	P value
BMI (kg/m²)	Between groups	308.92	3	102.97	1.59	2.68	0.19
	Within groups	7,794.98	121	64.42			
	Total	8,103.89	124				
WHR	Between groups	0.07	3	0.02	0.49	2.68	0.69
	Within groups	5.45	121	0.05			
	Total	5.52	124				
WC (cm)	Between groups	764.55	3	254.85	1.67	2.68	0.18
	Within groups	18,451.06	121	152.49			
	Total	19,215.61	124				
HC (cm)	Between groups	472.91	3	157.64	0.79	2.68	0.50
	Within groups	24,034.29	121	198.63			
	Total	24,507.20	124				
AC (cm)	Between groups	764.55	3	254.85	1.67	2.68	0.18
	Within groups	18,451.06	121	152.49			
	Total	19,215.61	124				

Notes: BMI – body mass index; WHR – waist-hip ratio; WC – waist circumference; HC – hip circumference; AC – abdominal circumference; df – degree of freedom
Source: compiled by the authors

Table 7. Relationship between anthropometric indices and age

Anthropometric indices	Correlation (r)	
	Male (N = 86)	Female (N = 125)
BMI	0.07	0.12
WHR	0.20	0.07
WC	0.11	0.23
HC	0.03	0.07

Notes: N – number of subjects; BMI – body mass index; WHR – waist-hip ratio; WC – waist circumference; HC – hip circumference
Source: compiled by the authors

The independent anthropometric indices studied were not influenced by age, as seen in this table. This could explain the reason for having lots of stroke cases even among the younger age group. Table 8 shows no correlation between age and the occurrence of stroke and other metabolic diseases for both male and female subjects.

Table 8. Relationship between age and occurrence of stroke and other metabolic diseases

Sex	Correlation (r)
Male	-0.03
Female	0.10

Source: compiled by the authors

The study therefore showed a very weak association with age, and rather holds the fact that the anthropometric indices are a stronger predictor of age. The anthropometric indices studied in the present work have shown a strong relationship with the occurrence of stroke in both male and female subjects. The study shows that these anthropometric indices are stronger stroke indicators than even age, which is a major non-modifiable risk factor in previous studies.

Discussion

A total of 211 participants were involved in the study, comprising 41% males and 59% females. The average age was 60.3 years for males and 57.5 years for females. Male subjects had an average BMI of 28.90 kg/m^2, while females had an average of 32.32 kg/m^2. The WHR was 0.94 for males and 0.95 for females, with male and female abdominal circumferences averaging 94.72 cm and 97.56 cm, respectively (Table 1). The study presents the mean height, weight, and BMI for different age groups of male subjects, which are shown in Table 2, ranging from 26 to 94 years. The mean BMI varied from 23.9 to 30.4, with four out of seven age groups classified as obese (>30) by the WHO BMI chart [13]. The remaining age groups were overweight (>24.9) or normal (18-24.9). Over 97% of male subjects had an overweight BMI, aligning with studies by M. Shiozawa *et al.* [14], indicating a link between abnormal BMI in males and the risk of stroke and other cardiovascular and metabolic diseases. There was an increase in the number of patients with an increase in abnormal BMI, such as over 18 subjects had a BMI of 30.43, 38 subjects had a BMI of 27 and 14 subjects had a BMI of 30.2. Further proving previous research such as that of M.A. Bashir *et al.* [15] who stated that obesity is a well-established stroke risk factor among Nigerian subjects and thus maintaining a healthy weight is crucial for stroke prevention. The present study thus observed very few numbers of patients with normal BMI values, such that only one patient had a BMI of 24.8 and two had a mean BMI value of 23.93. This proves BMI as a predictive marker for stroke and other metabolic diseases in the male subjects. Again, as seen in Table 2, the number of stroke cases increased with age up to 65 years. However, the decline in number of cases after 65 years cannot be explained by this study. The age group 66-75 has a relatively higher mean BMI, which, when combined with age, may indicate a higher risk of stroke. The greatest number of patients were documented at the ages of 56 to 65 years. This follows studies by F. Ramati *et al.* [16], who stated that stroke is a disease associated with ageing. This systematic review discovered that the prevalence of stroke increased with increasing mean age of the elderly and decreased with increasing year of publication. This non-modifiable risk factor increases the incidence of cerebrovascular events, doubling it for each decade after the age of 55 years, which is consistent with the findings for age groups 46-55 and 56-65 (Table 2). In further analysis, the present study obtained that no female subjects had a normal BMI. This suggests that 100% of female subjects had an overweight or obese BMI, consistent with prior research indicating a risk of stroke and other diseases associated with abnormal BMI in females [4, 6]. In the same vein, the greatest number of female patients were documented at the ages of 56 to 65 years (Table 2).

The data displays the mean WC, hip circumference, and WHR for different age groups of male subjects, as shown in Table 3. WHR ranged from 0.85 to 1.10, with six out of seven age groups having a low health risk WHR (<0.95) and one group having a high health risk WHR (>1.0). Approximately 99% of male subjects had a low health risk WHR (<0.95); however, the WHR value for the 99% of the subjects was high normal (within the range of 0.93 to 1.10). The higher the WHR value, the greater the number of subjects diagnosed with stroke and other metabolic disorders in male subjects. The present however shows that high normal WHR is a predictive marker for stroke and other metabolic diseases in male Nigerian subjects. These findings support previous research which suggest a link between abnormal WHR and increased health risks [9]. According to E. Taiwo & L.O.O. Thanni [8], WHR appears to be a more effective anthropometric parameter for predicting cardiovascular outcomes than BMI. Also, the mean WC tends to increase with age, with the highest value observed in the oldest age group (86-95). Increased WC is associated with abdominal obesity, which is a risk factor for stroke and cardiovascular diseases, supporting some previous studies [7, 8]. However, WC alone may not be suitable for comparing individuals with different body sizes and masses [8].

The mean HC also generally increases with age but begins a downward slope at ages greater than 55 in male subjects (Table 3). The present study shows that a larger number of male subjects diagnosed with stroke and other metabolic disorders had a greater HC, whereas only a few subjects with smaller HC were diagnosed with stroke and other metabolic diseases. Hence, HC is shown to be a good predictor of stroke and other metabolic disorders. However, in some isolated metabolic disorders like Type 2 diabetes mellitus, an inverse association exists between HC and the risk of developing diabetes in studies by A. Jayedi *et al.* [17]. Although these studies were cross-sectional, they consistently found inverse associations of HC with diabetes prevalence. It is worthy of note that more than 60% of subjects

in this present study had hypertension with less than 30% diagnosed with diabetes and stroke. It is thus imperative to say that most of the subjects with high HC fell into the category diagnosed with hypertension. Hence, it is correct to admit that the subjects in the present study had no inverse relationship between HC and other metabolic disorders because only a few of the subjects were diagnosed with diabetes. Therefore, finding an inverse relationship between HC and diabetes is justifiable. HC is thus a more complex predictive marker. Hence, making the WHR a more reliable metric according to Y. Li *et al.* [9]. The data shows variability in WC, HC, and WHR across age groups, emphasizing the importance of considering these factors collectively.

The data displays the mean waist and HC and WHR for different age groups of female subjects, as shown in Table 3. The mean WHR ranged from 0.83 to 1.01, with all age groups classified as high health risk (>0.80). This suggests that 100% of female subjects in this study had a high health risk WHR, in line with earlier studies showing a link between abnormal WHR in females and a risk of stroke and other metabolic illnesses [11]. The WHR values in the female subjects are generally lower than those of the male subjects, which is consistent with general findings for sex. Similar to the previous male data, WC tends to increase with age, with the highest values observed in the oldest age groups (76-85 and 86-95) (Table 3). Increased WC is associated with abdominal obesity, a known risk factor for stroke. These findings complement an additional report by A. Jayedi *et al.* [17].

Also, there is a statistically significant difference ($p = 0.00$) (Table 4) in the BMI between male and female subjects, indicating potential gender-related variations in body composition. The negative t-values suggest that, on average, males may have a lower BMI than females, as seen in the current study (Table 2). The significant differences in BMI between genders may contribute to variations in obesity-related risks for stroke and metabolic disorders. Central obesity is a known risk factor for these conditions. This is shown in the findings, females having greater BMI and more cases of stroke (Table 2). Again, ANOVA to test variation among age groups of male and female subjects (Tables 5 and 6) diagnosed with stroke and other metabolic disorders show that the anthropometric indices: BMI, WHR, WC, HC, and AC suggest no statistically significant differences ($p > 0.05$) between sexes. This infers that stroke and other metabolic diseases investigated in this study affects all age groups independently irrespective of their ages as long as their anthropometric indices are abnormal, especially the most predictive factors. Thus, all male and female subjects diagnosed with stroke and other metabolic diseases had similar anthropometric measurements, irrespective of their age differences. The present study also considered the relationship between anthropometric indices and age and discovered no correlation (r) between the two variables (Table 7). Therefore, age in this study had no much influence on the outcome of the anthropometric measures. This could also suggest the reason younger individuals with abnormal anthropometric indices had greater number of patients in the study. Although N. Ali *et al.* [18], in a study, stated that age is an independent non-modifiable risk factor for metabolic disorders, there was no correlation between age and the occurrence of stroke and other metabolic diseases in the present study (Table 8). Also, L. Li *et al.* [19] in a study documented a 67% increase in stroke incidence in participants younger than 55 years and a 15% decrease in participants 55 years and older. However, this seems to be in agreement with J. Kalita *et al.* [20] who reported higher stroke incidence in persons with greater economic challenges like unemployment and obtained more stroke occurrences in the males. It is imperative, as those younger than 55 years would fall into the class of people faced with joblessness and other economic issues. In a current study, however, S.L. Liew *et al.* [21] stated that brain age and not just the chronological age is a stronger risk factor and determines the outcome of post-stroke in stroke patients. The findings were taken from anthropological measurements of regions of the brain. This also is in consent with the present study which discovered that anthropometric indices: BMI, WHR and HC were better predictive markers than the age of the subjects.

Conclusions

The study provides valuable insights into the health profiles of male and female participants, revealing a high prevalence of abnormal BMI and WHR, with observation of gender-specific differences which should be noted during health interventions. Again, this study showed that males with high normal WHR were diagnosed with stroke and other metabolic disorders. Although WHR may not be an independent factor as it was not studied alone and more so, most of the participants in the present study had both an abnormal WHR ($F = 0.83$-1.01) and as well an abnormal BMI ($M = 27.7$-30.5, $F = 27.9$-33.6), a high normal WHR in males (0.93-1.10) could be a good predictor especially, as seen in this study. It is therefore imperative to note that individuals with normal but high-normal WHR should be high-risk indicators for stroke and other metabolic disorders. It is therefore noted that anthropometric indices like the BMI, WHR, WC and HC have great influence on the occurrence of stroke and other metabolic diseases. Again, though age is an important factor in previous studies, the current study shows that once the anthropometric indices are abnormal, age is not a major factor any longer. Hence, whether in young or old age, stroke, and other metabolic disorders could occur once there are abnormal anthropometric indices. This is important, therefore, because age and abnormal anthropometric indices are independently associated with stroke risk. It is important therefore for medical experts and caregivers to measure and document these anthropometric indices when managing patients with stroke and other metabolic diseases, as this could impact on the prognosis and more so predict future outcomes. It is also important that future studies should consider whether high normal WHR and/or even BMI are very strong indicators

for stroke. Studies looking at these anthropometric indices as independent risk factors for stroke and other metabolic diseases should be widely undertaken.

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Conflict of Interest

The authors declare no conflict of interest.

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Антропометричні показники як предиктори інсульту та інших метаболічних захворювань

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Анотація. Незважаючи на достатню кількість проведених досліджень щодо інсульту та його запобігання, він все ще залишається поширеною проблемою в Нігерії. Тому важливо виявити надійні та легкодоступні маркери для прогнозування інсульту та інших метаболічних захворювань. Відтак, дане дослідження було спрямоване на вивчення можливості прогнозування інсультів та інших метаболічних порушень за допомогою антропометричних показників. Досліджувана популяція включала 211 осіб, з них 86 чоловіків і 125 жінок у віковому діапазоні від 26 до 95 років. Відбір учасників здійснювався методом випадкової вибірки. Вимірювались такі антропометричні показники: індекс маси тіла, співвідношення обхвату талії до обхвату стегон, обхват талії, обхват стегон і обхват живота. Індекс маси тіла та співвідношення обхвату талії до обхвату стегон у жінок порівняно з чоловіками складали відповідно 32,32 і 0,95 для індексу маси тіла та 28,90 і 0,94 для співвідношення обхвату талії до обхвату стегон. У чоловіків спостерігали аномальний індекс маси тіла з піковими значеннями у віці старше 36 років. Найбільша кількість пацієнтів із інсультом та метаболічними захворюваннями була зафіксована у віці від 56 до 65 років як серед чоловіків, так і серед жінок. Усі жінки з діагнозом інсульту та інших метаболічних захворювань мали аномальне співвідношення обхвату талії до обхвату стегон (>0,80), тоді як більшість чоловіків мали високе, але нормальне співвідношення. Також спостерігалася статистично значуща різниця за статевою ознакою ($p \leq 0,05$) щодо індексу маси тіла та обхвату стегон у пацієнтів, із діагнозом інсульту та інших метаболічних захворювань Між віком, частотою інсульту та іншими метаболічними захворюваннями кореляції не виявлено. Результати дослідження свідчать про те, що антропометричні показники, такі як індекс маси тіла, співвідношення обхвату талії до обхвату стегон та обхват стегон є кращими прогностичними маркерами, ніж вік досліджуваних осіб. Також дослідження показало, що високе, але нормальне співвідношення обхвату талії до обхвату стегон є гарним маркером для прогнозування інсультів та інших метаболічних розладів у чоловіків. Отримані дані будуть корисними для медичних фахівців, дієтологів та асоціацій з профілактики інсульту

Ключові слова: антропометрія; індекс маси тіла; серцево-судинні захворювання; обхват стегон; співвідношення обхвату талії до обхвату стегон

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Antifungal susceptibility and speciation of *Candida* isolated from blood at a tertiary care centre

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Abstract. There has been a rise in the incidence and prevalence of fungal infections worldwide, especially by *Candida* spp. leading to significant morbidity and mortality. Early recognition of *Candida* bloodstream infection has been associated with improved outcome in patient care. Hence, the present study was carried out to determine the distribution of the *Candida* species that causes candidemia as well as its antifungal susceptibility pattern in the hospital. A total of 8,087 blood cultures received from various clinical departments of a tertiary care centre were processed via the Automated blood culture system BACTEC FX40 or manually as per standard protocol in the Department of Microbiology from January to December 2022. Isolated *Candida* spp. were identified using biochemical tests and CHROM agar. Antifungal susceptibility was performed and interpreted as per Clinical and Laboratory Standards Institute guidelines. A total of 2,010 blood cultures showed a positive culture growth of microorganisms, out of which, *Candida* spp. was isolated in 123 blood cultures (6.11%). The Neonatal Intensive Care Unit accounted for the isolation of 78.8% of *Candida* spp. *C. krusei* was found to be the most common isolate 36.5% followed by *C. albicans* (21.2%), *C. glabrata* (19.5%), *C. parapsilosis* (13.8%) and *C. tropicalis* (9%). Voriconazole was found to be the most effective antifungal agent, with 81.3% of *Candida* spp. showing susceptibility to it, and was found to be the most effective antifungal agent. Non-albicans *Candida* spp., *C. krusei* was found to be the predominant isolate in the present study. The neonatal age group was the most commonly affected age group in candidemia. It is advisable to monitor the changing trend of *Candida* species in particular, geographical

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area to get an idea about prevalent species and their antifungal susceptibility pattern for choosing empirical therapy and better patient management

Keywords: candidemia; *Candida* non-albicans; antifungal drug sensitivity; voriconazole

Introduction

Fungi have emerged as a major public health concern since the late 20th century and have significantly increased the morbidity and mortality of patients admitted in intensive care units (ICU) and among immunocompromised patients [1]. Increased incidence of candidemia over the years is largely due to advancements in medical interventions, numerous elderly and susceptible population having transplantation and haematological malignancies [2]. According to data from the Centers for Disease Control (CDC), *Candida albicans* is the eighth most prevalent nosocomial infection [1]. As mentioned in the study, the National Nosocomial Infection Surveillance (NNIS) system of the United States, has stated *Candida* as the fourth common pathogen causing nosocomial bloodstream infections (BSI).The international EPIC II study, as quoted by M. Schroeder *et al.* [3], has shown *Candida* to be the third most common source of infection in over 14,000 patients; patients with *Candida* bloodstream infection had a significantly greater mortality rate than those with Gram-positive or Gram-negative bloodstream infections.

Even though *Candida albicans* is thought to be the most common cause of candidemia, since the 1980s, there has been an upsurge in infections brought on by other *Candida* species [3-5]. Since Non-albicans *Candida* (NAC) species frequently exhibit intrinsic and/or acquired resistance to widely used antifungal medications, the advent of NAC is a serious concern. Antifungal medication is now widely used as a preventative measure in high-risk patients suspected of having invasive and systemic candidiasis. Acquired antifungal resistance in *Candida* varies greatly when it comes to antifungal medicines. Reports of a fluconazole-resistant *C. glabrata* and *C. parapsilosis* outbreak are already available [6]. This justifies the need of the speciation of *Candida* isolates for formulating guidelines for empirical therapy at the local level. B. Dalyan Cilo [7] and M. Carbia *et al.* [8] have reported that the range of agents associated with candidemia differs throughout nations, years, even hospitals within the same nation. Because of this, it's critical to perform surveillance studies on a regular basis to track changes in the aetiology and susceptibility pattern related to the treatment of fungal infections in hospitals.

India is a developing country with more than 1.3 billion population, hot and humid weather in most of the states throughout the year, liberal use of over-the-counter antibiotics and steroid, a large pool of undiagnosed/poorly controlled diabetes and other immunocompromised status, so it becomes necessary to have a baseline data for each region [1]. A review of literature suggests that it is better to have baseline data of the local geographical area for better patient care. As scant data relating to candidemia is available for Central Gujarat, the present study was undertaken to determine the prevalence of candidemia, species distribution and their antifungal susceptibility pattern as blood isolates was taken up.

Materials and Methods

The study was conducted in Central Gujarat, India, at a tertiary care teaching hospital's Department of Microbiology from January 2022 to December 2022. Throughout this time, the hospital received and processed 8,087 blood culture samples from patients who had been hospitalised to different clinical departments with suspected septicemia or pyrexia of unknown cause via Automated blood culture system BD BACTECFX40 (BD diagnostics, USA) or by conventional manual techniques as per the standard procedures [9]. All the blood culture bottles received in the department with adequate volume and proper labelling of patient demographic details from suspected sepsis, irrespective of age, sex and clinical wards were included in the study. Positive flagged blood culture bottles and/or conventionally processed blood culture bottles were then subcultured on Brain heart infusion agar (Microexpress, A division of Tulip Diagnostics Pvt. Ltd, India) and MacConkey agar (Microexpress, A division of Tulip Diagnostics Pvt. Ltd, India.). The inoculated plates were incubated at 37°C for 24 hours. Conventionally processed blood culture bottles were sub-cultured on alternate day to look for growth and incubated maximum for 7 days at 37°C. Automated blood culture system BD BACTEC FX40 processed blood cultures which flagged green at the end of 5 days of incubation were reported negative. Creamy, smooth, pasty and convex colonies on Brain heart infusion agar were subjected to Gram stain. Gram-positive budding yeast-like cells presumptively identified as *Candida* spp. were further confirmedly performing a Germ tube test, the colour of the colony on CHROM agar (Hi-Medial Pvt.Ltd, India), Slide culture on Corn meal agar (Microexpress, A division of Tulip Diagnostics Pvt. Ltd, India) and sugar fermentation test.

For Germ tube test, 500µL of serum was inoculated with a loopful of *Candida* colonies from Brain heart infusion agar and incubated for 2 hours at 37°C. The presence of germ tube was considered as *Candida albicans* isolate. The absence of germ tube development was considered as *Candida* non-albicans species. For further speciation, the colony was cultured on CHROM agar and a different colour for *Candida* spp. was recorded and interpreted as per the manufacturer's instructions. Slide culture on Corn meal agar (Microexpress, A division of Tulip Diagnostics Pvt.Ltd, India.) was also performed for morphological identification and co-relation. Antifungal susceptibility testing by E-strip was carried out on Muller Hinton Agar (Microexpress, A division of Tulip Diagnostics Pvt.Ltd,

India) with added 2% Glucose and 0.5 µg/mL Methylene blue. Five millilitres of sterile saline were used to suspend four to five colonies that were picked up. After vortexing the suspension, the turbidity was brought down to 0.5 McFarland levels. Using a sterile brush dipped in the suspension, lawn culture was performed after the incubation period of approximately 15 to 20 minutes. Antifungal susceptibility was carried out by E strips (Hi-media Pvt. Ltd., India) for Voriconazole (0.002-32 µg/mL), Fluconazole (0.016-256 µg/mL), Ketoconazole (0.002-32 µg/mL) and Amphotericin B (0.002-32 µg/mL). Disc diffusion susceptibility was tested for Clotrimazole (10 µg) and Nystatin (50 µg). The result was interpreted as susceptible, resistant, intermediate or susceptible dose-dependent per CLSI M27M44S [10] and EUCAST [11] guidelines.

Patients' demographic data, place of admission & the laboratory findings were entered in Microsoft Excel for frequency distribution analysis. The Chi-square test was applied for categorical data and statistical significance was studied using Statulator, an online calculator for analysis and interpretation of result [12]. p-value of <0.05 was considered as the statistical significance and the association was established for that factor. Only clinical samples received by the laboratory for routine analysis were included in the study, with no direct patient involvement. All the data collected were from patients' requisition forms received along with the samples and LIS software of the hospital. Every method used in research with human participants conforms with ethical guidelines [13, 14].

Results

Of 8,087 blood culture samples received in the Laboratory, 2,010 samples (24.85%) were found to be culture positive for microorganisms. *Candida* spp. were isolated in 123 samples (6.11%) from these positive blood culture samples as shown in Figure 1. The bacterial aetiology was not evaluated for present study analysis. On an average, the blood culture took 48-72 hours to be positive for *Candida* growth.

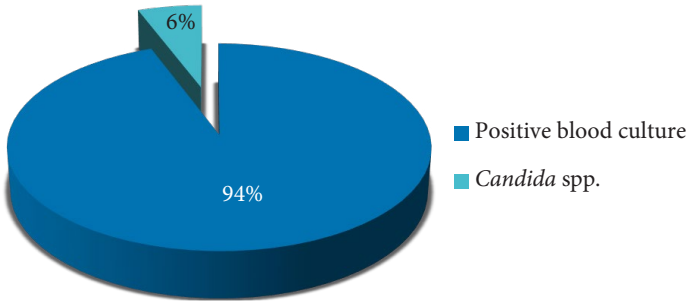

Figure 1. % *Candida* spp. isolated from positive blood culture samples
Source: compiled by the authors

Among the 123 *Candida* isolates, the majority of the *Candida* isolates were from the Neonatal Intensive Care Unit and baby room, being, 97 isolates (78.86%) followed by the Paediatric ward and Paediatric ICU 13 isolates (10.5%), Medicine wards & ICU had 10 isolates (10.5%) and 3 (1.6%) from others as depicted in Figure 2.

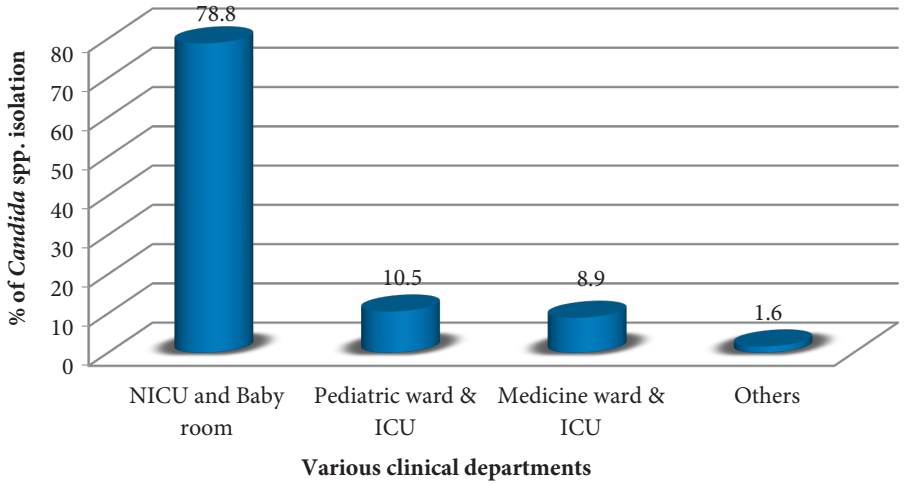

Figure 2. % Distribution of *Candida* isolates from the various clinical departments
Notes: NICU – Neonatal Intensive Care Unit; ICU – Intensive Care Units
Source: compiled by the authors

Analysis of patients' age group-wise distribution showed 102 patients (83%) in <1 year of age group, 7 patients (5.7%) in 1-10 years of age, 11 patients (8.9%) in 30-45 years of age and 3 patients (2.4%) in >60 years of age. Microbiological data analysis was based on the results from culture on CHROM agar, corn meal agar morphology, germ tube and sugar fermentation tests. Based on the different colour production by different species of *Candida* on CHROM agar *Candida* spp. were identified as *C. albicans* (dark green), *C. tropicalis* (dark blue), *C. krusei* (pale pink), *C. parapsislosis* (white to pale pink) and *C. glabrata* (white). Chlamydospore formation, arrangements of conidia and/or pseudohyphae on slide culture on corn meal agar confirm the *Candida* speciation. From all the morphological and biochemical analysis, 97 isolates (78.8%) were *Candida* non-albicans and 26 isolates (21.2%) were *C. albicans*. Over all *Candida* species isolated were *C. krusei* (36.5%), *C. albicans* (21.2%), *C. glabrata* (19.5%), *C. parapsilosis* (13.8%) and *C. tropicalis* (9%). The distribution of various *Candida* spp. is shown in Figure 3.

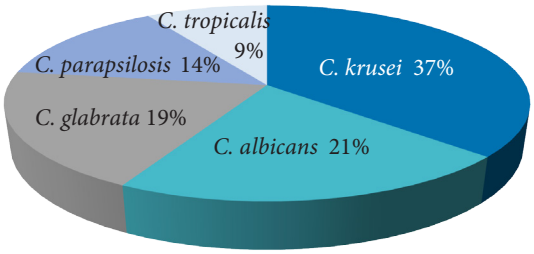

Figure 3. % Distribution of various *Candida* spp.
Source: compiled by the authors

Of the total 123 *Candida* isolates, 84 (68.3%) were from ICU settings and 39 (31.7%) were from ward settings. The speciation of *Candida* isolates from ICU showed a predominance of *Candida* non-albicans, i.e. 66 (78.6%) isolates and 18 (21.4%) as *Candida albicans*. Similarly, 31 (79.5%) were *Candida* non-albicans and 8 (20.5%) were *Candida albicans* in Wards' isolates. The Chi-square test using Statulator, an online calculator, showed a p-value of 0.75 for this comparison, which was not found to be statistically significant. 70 *Candida* isolates (56.9%) were recovered from male patients, while 53 isolates (43.1%) were from female patients. Amongst 70 isolates from men, 56 (80%) were *Candida* non-albicans and 14 (20%) were *Candida albicans*. Of the 53 isolates from women, 41 (77.3%) were *Candida* non-albicans while 12 (22.6%) were *Candida albicans*. The chi-square test using Statulator, an online calculator showed a p-value of 0.81 for this comparison, which is statistically not significant suggesting *Candida* speciation, has no association with the gender of patient. Antifungal susceptibility testing result observed in all *Candida* isolates of the present study is shown in Figure 4.

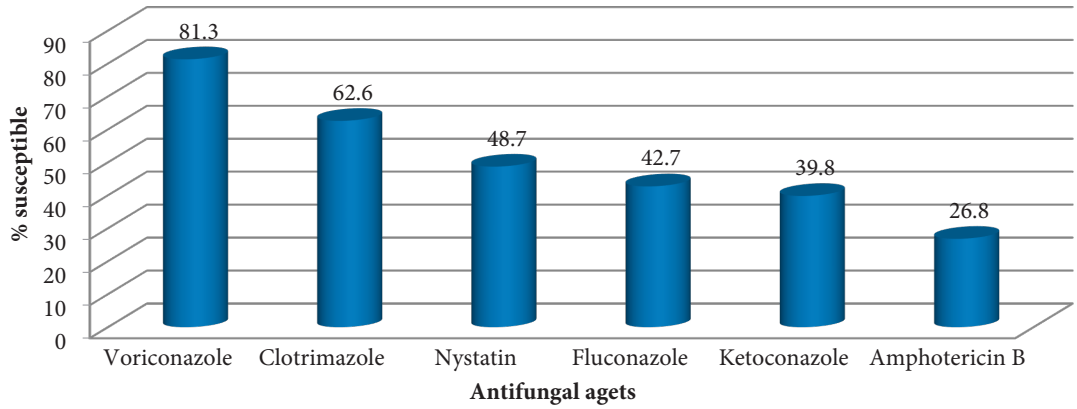

Figure 4. % Susceptibility of all *Candida* isolates to various antifungal agents
Source: compiled by the authors

The overall susceptibility pattern of *Candida* isolates showed 81% susceptibility to Voriconazole followed by 62% Clotrimazole. Least susceptibility was observed to Amphotericin B. Antifungal susceptibility pattern in the *C. krusei* isolates was also evaluated, where 73.3% susceptibility was observed in Voriconazole followed by 66.66% in Clotrimazole and 28.8% to Nystatin. The high degree of resistance to almost all the azoles suggested intrinsic resistance in the species. Amongst *C. albicans* isolates, the highest susceptibility was observed in Voriconazole (84.6%) followed

by Fluconazole and Clotrimazole (57.6%), Ketoconazole (46.1%), Nystatin (42%) and Amphotericin B (38.4%). The average MIC for Voriconazole ranged from 0.016-0.032 and from 0.50-1 for Fluconazole.

To summarise, the culture positivity rate due to candidemia was 6.11%. A majority of the *Candida* isolates,78.86% were from Neonatal ICU and baby room. 56.9% of the *Candida* isolates from male patients, with male predominance being observed in the present study. 78.8% isolates were *Candida* non-albicans, *C. krusei* being the most common. Overall, 81.1% isolates were susceptible to Voriconazole. As the data collection was from requisition forms mainly, detailed clinical history of patients, risk factors, co-morbid conditions, average hospital stay, and mortality rate were not evaluated in present study.

Discussion

A common problem for patients admitted to tertiary care facilities is fungal infections. There has been an increase in *Candida* spp. infections over the last 40 years, especially those brought on by *Candida* non-albicans spp. Because of this, the laboratory diagnosis is essential for accurately identifying the species at issue and starting prompt, effective therapy for patients. The prevalence of candidemia in developing countries is reported less because of limited studies in such settings. Even with such limited data, developing countries have reported 4-15 times higher rate of candidemia than developed nation [2].

Table 1 depicts the comparison of the present study with other studies from Indian authors and authors from across the globe.

Table 1. Comparison of present study data with other studies

Study by	Study region	Study duration	Rate of candidemia	Sex predominance	*Candida* speciation
S. Ahmad *et al.* [1]	UP, India	2018-2019	-	Male – 53.3%	*C.albicans* – 28% *C.tropicalis* – 49% *C.parapsiolosis* – 10.4%
M. Schroeder *et al.* [3]	Germany	2008-2017	0.5%	Male – 61.4%	*C.albicans* – 60.9% *C.glabrata* – 19.4% *C.parapsiolosis* – 6.6%
E. Rajni *et al.* [4]	Rajasthan India	2017-2020	2.8%	M:F=2:1	*C.albicans* – 11% *C.tropicalis* – 38% *C.parapsiolosis* – 18%
E.J. Kim *et al.* [15]	Korea	2006-2017	2.4%	-	*C.albicans* – 39.9% *C.tropicalis* – 20.2% *C.parapsiolosis* – 18.2%
E. Ghrenassia *et al.* [16]	France	2002-2017	0.7%	Male – 61%	*C.albicans* – 54% *C.glabrata* – 19%
C. Agnelli *et al.* [17]	Brazil & Spain	2010-2018	-	Male – 57.1%	*C.albicans* – 45.4% *C.parapsiolosis* – 20.8% *C.glabrata* – 14.2%
S. Mazzanti *et al.* [18]	Italy	2010-2018	2.2%	Male – 62%	*C.albicans* – 52% *C.parapsiolosis* – 24% *C.glabrata* – 14%
N. Alkharashi *et al.* [19]	Saudi Arabia	2008-2015	-	Male – 53.4%	*C.albicans* – 33% *C.tropicalis* – 22.2% *C.glabrata* – 18.5%
E.E. Ricotta *et al.* [20]	USA	2009-2017	-	Male – 51%	*C.albicans* – 48% *C.glabrata* – 24% *C.parapsiolosis* – 11%
D. Solomon *et al.* [21]	Kenya	2019-2020	8.2%	Male – 58%	*C.auris* – 29% *C.albicans* – 25.8% *C.parapsiolosis* – 19.3%
Present study	Central Gujarat, India	2022	6.11%	Male – 56.9%	*C.albicans* – 21.2%, *C.krusei* – 36.5%, *C.glabrata* – 19.5%

Source: compiled by the authors

The higher rate of candidemia in the present study might be due to lesser study duration as compared to the other studies, where they have conducted the studies for 3-15 years. Agneli *et al.* [17] and N. Alkharashi *et al.* [19] have just mentioned the *Candida* isolates included in their study, so the rate of candidemia was not calculated. A larger study duration gives a better idea about the change in the rate of candidemia over the years and the prevalence of common species over time. T.S. Al-Musawi *et al.* [22], H. Chawda *et al.* [23] and W. Alkhalifa *et al.* [24] have also reported more cases of candidemia in male patient. All the studies reviewed here show that candidemia is more reported in male patients all over the world. Thus, the present study's finding about male preponderance is well correlated.

78.8% of isolates in the present study were from the NICU and baby room. This finding is similar to the other study from Gujarat by H. Chawda *et al.* [23] who reported 98% isolates from NICU. N. Alkharashi *et al.* [19] have reported 67.6% and E. Rajni *et al.* [4] have reported 78.9% isolates from ICU. S. Sridharan *et al.* [25] have reported that 89.5% candidemia patient in their study had ICU stay. The results of the present study demonstrate that candidemia is a serious problem in intensive care units. This would be linked to advances in organ support systems, overuse of antibiotics, and improved diagnostics, which lengthens ICU stays [1]. The present study area being a tertiary care setting supports this observation.

The most frequent cause of candidemia in this investigation was *Candida* non-albicans, which is consistent with the results of other Indian studies that are included in the table 1 as well as studies by H. Chawda *et al.* [23] and W. Alkhalifa *et al.* [24]. S. Boonsilp *et al.* [26], the authors reported in their study from Thailand that *C. tropicalis* as the most common isolate in candidemia patients. On the contrary, European studies by M. Schroeder *et al.* [3], E. Ghrenassia *et al.* [16] and S. Mazzanti *et al.* [18], found *Candida albicans* predominant isolate. A study by E. Lindberg *et al.* [27] at a Swedish hospital has reported *Candida albicans* as the most common species. N. Alkharashi *et al.* [19] and T.S. Al-Musawi *et al.* [22] from Saudi Arabia also have noted *Candida albicans* as a major isolate.

The most prevalent *Candida* non-albicans isolate in the current investigation was *C. krusei*, which was followed by *C. glabrata*, *C. parasitosis*, and *C. tropicalalis*. A study in west Gujarat by H. Chawda *et al.* [23] has reported *C. tropicalis* as the most common species, followed by *C. glabrata*, *C. gullermondii* and *C. parapsilosis*. A North Indian study by H. Kaur *et al.* [2] has observed *C. tropicalis* as the most common species followed by *C. pelliculosa* and *C. krusei*. In a Korean study by E.J. Kim *et al.* [15], the authors have reported *C. tropicalis* to be the most common, *Candida* non-albicans followed by *C. parapsilosis*. These differences point out that different species are predominant in different geographical area, thus making it mandatory for *Candida* speciation for local data.

In the present study, 81.3% susceptibility to Voriconazole was found, followed by 62.6% to Clotrimazole, 48.7% to Nystatin and 42.7% to Fluconazole. N. Alkharashi *et al.* [19] have noted 89% susceptibility to Voriconazole and 60% to Fluconazole in *Candida* non-albicans isolates. S. Ahmad *et al.* [1], in their study from UP, India, have observed susceptibility of 80% to Voriconazole, 67% Fluconazole, 62% to Amphotericin B and 14% to Nystatin. E. Ghrenassia *et al.* [16] have shown 70% susceptibility to Fluconazole and 92% to Echinocandins. H. Chawda *et al.* [23] have reported 100% sensitivity to Voriconazole, 98.8% to Fluconazole, 73% to Ketoconazole and 68.6% to Clotrimazole. So, if evaluation of the susceptibility data of various antifungal drugs is made, there is a constant increase in the resistance is reported. Therefore, it is important to monitor the change in susceptibility pattern over the years.

Conclusions

At the conclusion of the study, the primary goal of the investigation was to assess the antifungal susceptibility pattern and profile of *Candida* spp. isolated from blood culture in different wards and intensive care units, which was achieved. As per the study result, *Candida* non-albicans was the most common agent causing Candidemia and *C. krusei* was found to be the most common species. The culture positivity rate for candidemia was 6.11%, indicating a significant presence of this infection among the blood culture samples. The majority of *Candida* isolates (78.86%) were from the Neonatal Intensive Care Unit and baby room, indicating a higher vulnerability in this age group. Specifically, 83% of the patients with candidemia were less than 1 year old. Being a tertiary care teaching Hospital catering to critical patients from rural area and adjacent states, ICU setting has a maximum of *Candida* spp. isolation. A male predominance was observed, with 56.9% of the *Candida* isolates being recovered from male patients. Voriconazole was the most effective antifungal drug in the present study area, with more than 80% susceptibility. Therefore, in order to better treat candidemia infections, the current study highlights the importance of routine monitoring investigations in all institutions. This will raise public knowledge of candidemia and its risk factors and provide details regarding the potential long-term effectiveness of community-distributed antifungals. The study identified gaps in data, such as the lack of evaluation of biofilm formation, patient outcomes, risk factors, and detailed clinical histories. Future research should aim to address these aspects to provide a more comprehensive understanding of candidemia and its management. Further studies incorporating these factors will help in formulating more effective treatment protocols and preventive measures.

Acknowledgements

None.

Conflict of Interest

The authors declare no conflict of interest.

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Чутливість до протигрибкових засобів і видова ідентифікація грибків роду *Candida*, ізольованих з крові в третинному медичному центрі

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Анотація. У світі спостерігається зростання захворюваності та поширеності грибкових інфекцій, особливо зумовлених *Candida* spp., що призводить до значної захворюваності та смертності. Раннє виявлення кандидемії було пов'язане з покращенням результатів лікування пацієнтів. Таким чином, дане дослідження було проведено для визначення розподілу видів *Candida*, які викликають кандидемію, а також їхнього профілю чутливості до протигрибкових препаратів у лікарні. Загалом 8,087 гемокультур, отриманих з різних клінічних відділень третинного медичного центру, були оброблені за допомогою автоматизованої системи BACTEC FX40 або вручну за стандартним протоколом у відділенні мікробіології з січня по грудень 2022 року. Виділені *Candida* spp. були ідентифіковані за допомогою біохімічних тестів на хромогенних середовищах. Чутливість до протигрибкових препаратів проводили та інтерпретували відповідно до рекомендацій Інституту клінічних та лабораторних стандартів. Загалом 2,010 гемокультур показали позитивний ріст мікроорганізмів, з яких у 123 культурах (6,11 %) були виділені *Candida* spp. 78,8 % *Candida* spp. були виділені з відділення неонатальної інтенсивної терапії. C. *krusei* була найпоширенішим ізолятом (36,5 %), далі C. *albicans* (21,2 %), C. *glabrata* (19,5 %), C. *parapsilosis* (13,8 %) та C. *tropicalis* (9 %). Серед виділених штамів *Candida* spp. 81,3 % були чутливими до вориконазолу, який виявився найефективнішим протигрибковим препаратом. Серед неальбіканс штамів *Candida* spp., C. *krusei* була найпоширенішим ізолятом у даному дослідженні. Кандидемія найчастіше виявлялася у неонатальній віковій групі. Доцільно відстежувати тенденцію зміни видів *Candida* у конкретному географічному регіоні, щоб отримати уявлення про поширені види та їхній профіль чутливості до протигрибкових препаратів для вибору емпіричної терапії та кращого ведення пацієнтів

Ключові слова: кандидемія; неальбіканс *Candida*; чутливість до протигрибкових препаратів; вориконазол

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Comparison of the effectiveness of training methods for medical practitioners in Ukraine regarding anaphylaxis

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Abstract. The incidence of anaphylaxis is increasing worldwide. Therefore, it is crucial for medical practitioners to be aware of anaphylaxis symptoms and respond effectively. The purpose of this study was to assess the level of Ukrainian doctors' knowledge regarding anaphylaxis, ability to recognise variable presentations of anaphylaxis, and knowledge of adrenaline administration depending on their form of training. A cross-sectional study was conducted between May 2023 and June 2023, involving physicians from different specialities, medical paramedics, medical students, and interns in Ukraine. A standardised anonymous questionnaire based on the European Academy of Allergy and Clinical Immunology guidelines for anaphylaxis (2021 update) was used to evaluate knowledge of clinical criteria for diagnosing and managing anaphylaxis. The study compared two groups: one with completed European Resuscitation Council courses in simulation centres (Group 1) and the other without such practical reinforcement (Group 2). Respondents primarily acquired knowledge about anaphylaxis during university studies and from Ukrainian guidelines, with only a minority referring to international guidelines. Approximately half of the participants completed European Resuscitation Council courses in simulation centres, suggesting the potential positive impact of practical reinforcement of theoretical knowledge. Group 1 demonstrated a higher percentage of recognising anaphylaxis in scenarios that combined symptoms of the respiratory and gastrointestinal systems without involving mucosal tissue and skin manifestations after exposure to a potential allergen, and they also provided more accurate responses regarding the route of adrenaline administration. Nonetheless, there was also a tendency for overdiagnosis by respondents in Group 1. The study revealed differences in the diagnosis and management of anaphylaxis among medical practitioners, with an advantage for those who completed European Resuscitation Council courses. Continuous education and simulation-based training are crucial for reducing anaphylaxis mortality and improving treatment outcomes

Keywords: anaphylaxis; knowledge; physicians; adrenaline; training

Introduction

In recent years, the global incidence of all-cause anaphylaxis has been on the rise, primarily driven by reactions to medications and food [1]. Current data indicate a worldwide incidence of anaphylaxis ranging from 50 to 112 episodes per 100,000 person-years, with an estimated lifetime prevalence between 0.3% and 5.1%. These figures vary depending on the definitions used, study methodologies, and geographical locations [2]. The lack of accurate anaphylaxis diagnoses and suboptimal management practices contribute to significant public health challenges [3].

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Despite the rising occurrence of anaphylaxis, numerous instances have gone unnoticed or unreported. The study by M. Cuevas *et al.* [4] established a significant correlation between the administration of adrenaline and the improvement in outcomes in the ground-based emergency cohort. M.S. Shaker *et al.* [5] mentioned that anaphylaxis, presenting various clinical manifestations, takes place in doctor practice of any speciality. It is crucial to recognise this condition and know how to act due to a patient's life depends on the correctness and immediacy of the actions of doctors. Practical experiences not only solidified their understanding of medical concepts but also enhanced their ability to apply that knowledge in real-life scenarios, leading to more accurate diagnoses and treatment plans [6].

Earlier research uncovered the challenges faced by healthcare professionals in precisely recognising anaphylaxis and providing appropriate treatment. The study by S. Cimen Sipahi & S.B. Sayili [7] noted that just 66.7% of participant of cross-sectional survey indicate the correct dose of epinephrine. The administered questionnaire was conducted in Al-Qassim, Saudi Arabia by H.N. Alsaleem *et al.* [8]. According to it, the average knowledge score of primary healthcare physicians was 4.74 out of a total of 10 points. Almost half (48.8%) had poor knowledge levels, 43% had moderate knowledge levels, and only 8.3% had good knowledge levels. According to the data of M. Cuevas *et al.* [4] study, guideline-compliant first-line therapy with adrenaline was not administered in the majority of cases analysed in this study. The significant impact of adrenaline on outcomes highlighted in this study underscores the need to enhance the emergency treatment of anaphylactic reactions. Epinephrine stands as the primary drug in addressing anaphylaxis, and various earlier studies indicate a deficiency in understanding its prescribed dosage and administration route. Ambiguity persists in choosing the initial treatment line. The purpose of this study was to assess the level of Ukrainian doctors' knowledge regarding anaphylaxis, ability to recognise variable presentations of anaphylaxis, and knowledge of adrenaline administration depending on their form of training.

Materials and Methods

This cross-sectional study was conducted between May 2023 and June 2023 in the simulation centre TESIMED of the I. Horbachevsky Ternopil National Medical University, Ukraine. A total of 175 medical practitioners from different part of Ukraine took part in the survey. To further explore the impact of simulation-based training, the knowledge of diagnosis and treatment of anaphylaxis was evaluated and compared between two groups. Group 1 consisted of 89 medical practitioners who completed ERC courses in simulation centres, while Group 2 comprised 86 respondents who did not practice in such centres.

An anonymous questionnaire based on the European Academy of Allergy and Clinical Immunology (EAACI) guidelines [9] for anaphylaxis (2021 update) was employed to evaluate their knowledge of clinical criteria for diagnosing and treating anaphylaxis. The questionnaire consisted of 18 multiple-choice questions designed to assess respondents' ability to identify the true manifestations of anaphylaxis, including possible combinations of affected systems and their relationship with allergen exposure. Participants were required to recognise key clinical features such as the rapid onset of symptoms (ranging from minutes to several hours), involvement of the skin, mucosal tissues, or both, and signs of respiratory distress or reduced blood pressure (BP) along with associated symptoms of end-organ dysfunction. They also needed to identify reduced BP following exposure to a known allergen and recognise respiratory compromise or persistent gastrointestinal symptoms after exposure to a likely allergen for that individual. In addition, they must know about the route and the knowledge regarding the timing of the second dose of adrenaline administration. It was decided to compare the knowledge of medical practitioners from 12 cities in Ukraine who completed European Resuscitation Council (ERC) courses such as Advanced Life Support (ALS), European Paediatric Advanced Life Support (EPALS), with those who had not had such experience. Permission for the processing and publication of the collected information was obtained from every respondent via Google Form. The information from individuals was collected without revealing their identity. A graphic guide on the criteria for the diagnosis of anaphylaxis, based on the EAACI guidelines [9] developed by the author of this manuscript in the Ukrainian language, was distributed to those who responded to the given anonymous questionnaire automatically after submission of Google form. According to excerpt from Protocol No. 75 (01.11.2023) of the meeting of the Bioethics Commission of I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine, the study meets the requirements of norms and principles of bioethics. The study also adheres to the principles of the Declaration of Helsinki, ensuring ethical conduct and the protection of participants' rights and well-being throughout the research process [10]. Descriptive statistics (group-wise percentages) was performed using MSExcel 2000 software suite to compare the level of knowledge between the different groups. Chi-square test was used to assess the differences and statistical significance was defined as a p value <0.05.

Results

Regarding the sources of their knowledge, a majority of the respondents pointed to their university education (101 respondents, 57.7%) and Ukrainian guidelines (87 respondents, 49.7%) as the main contributors to their understanding of anaphylaxis diagnosis and treatment. However, only a minority of respondents (36 or 20.5%) relied on international guidelines, possibly suggesting the need for greater dissemination and incorporation of international best practices into Ukrainian medical education. Of note, 89 respondents (50.8%) had completed ERC courses in simulation centre.

In general, respondents demonstrated a relatively high level of correct diagnosis for anaphylaxis scenarios involving mucosal tissue in combination with hypotension (119

of all respondents – 68%) or respiratory failure (132 of all respondents – 75.4%). The comparison between the two groups revealed only minor differences in their responses to these scenarios (Fig. 1).

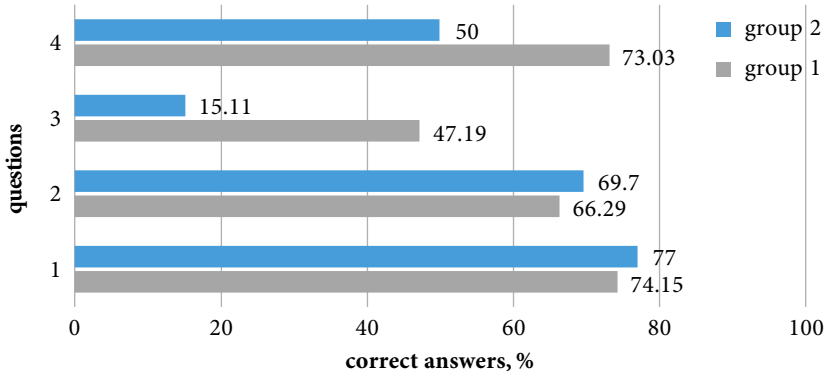

Figure 1. Correct answers about clinical criteria for diagnosing anaphylaxis, %
Notes: 1. acute onset of an illness with involvement of the skin/ mucosal tissue and respiratory compromise; 2. acute onset of an illness with involvement of the skin/ mucosal tissue and reduced BP; 3. respiratory compromise + persistent gastrointestinal symptoms after exposure to a likely allergen for that patient; 4. reduced BP after exposure to known allergen for that patient
Source: compiled by the authors

Group 1 (ERC course completion) exhibited a higher rate of correct diagnosis in case of reduced blood pressure after exposure to a known allergen for that patient (65 respondents, 73.0%) ($p < 0.05$) compared to Group 2 (43 respondents, 50.0%). However, both groups showed a similar rate of incorrect diagnoses, with 118 of all respondents (67.4%) mistaking angioedema for anaphylaxis (59 persons, 66.0% in Group 1 vs. 59 persons, 68.6% in Group 2).

There was a substantial difference in recognising anaphylaxis with respiratory compromise and persistent gastrointestinal symptoms without involvement of skin-mucosal tissue after exposure to a likely allergen for that patient. Group 1 respondents demonstrated a higher rate of correct recognition (42 persons, 47.19%) ($p < 0.05$), whereas only 13 (15.11%) ($p < 0.05$) of Group 2 respondents answered correctly.

Nonetheless, there was also a tendency for overdiagnosis by respondents in Group 1, as 45 (50.56%) of them recognised anaphylaxis in scenarios involving skin and gastrointestinal tract symptoms without information about exposure to a likely allergen for that patient (Fig. 2).

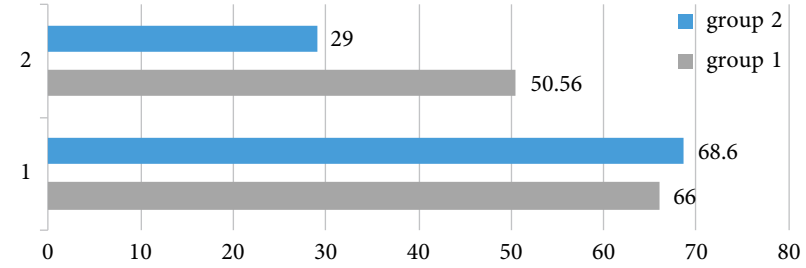

Figure 2. Wrong answers about clinical criteria for diagnosing anaphylaxis, %
Notes: 1. angioedema; 2. vomiting and generalised hived without exposure to a likely allergen for that patient
Source: compiled by the authors

Regarding the treatment of anaphylaxis, the majority of medical practitioners (133.76%) preferred the intramuscular (IM) route for adrenaline administration. Group 1 respondents demonstrated better knowledge of the correct route, with 79 (88.76%) ($p < 0.05$) selecting IM, compared to 53 (61.63%) in Group 2. 129 (73.7%) of the medical practitioners indicated the right site of IM (among respondents of Group 1 – 77 (86.51%) ($p < 0.05$), among respondents of Group 2 – 52 (60.40%). Nevertheless, some misconceptions were observed, as 16 (9.1%) of all respondents mistakenly believed that adrenaline should be administered subcutaneously (SC) (among respondents of Group 1 – 3 (3.37%), among respondents of Group 2 – 13 (15.11%)), and 26 (14.9%) wrongly selected the intravenous route (IV) (among respondents of Group 1 – 7 (7.86%), among respondents of Group 2 – 20 (23.25%)) (Table 1).

Table 1. Adrenaline administration route

Variants of answers	Group 1		Group 2	
IM	79	88.76%	53	61.63%
IV	7	7.86%	20	23.25%
SC	3	3.37%	13	15.11%

Notes: IM – intramuscular; IV – intravenous; SC – subcutaneous
Source: compiled by the authors

The minimal timing of possible repeating adrenaline administration is 5 minutes after the first dose if there was no improvement or worsening of symptoms [11]. The majority of respondents (119, 68%) accurately indicated this timing. Notably, Group 1 respondents displayed better understanding, with 74 (83.15%) ($p < 0.05$) providing the correct response, compared to 45 (52.32%) in Group 2. Some respondents (25, 14.3%) erroneously noted that the second dose should be administered just after 20 minutes: among respondents of Group 1 – 7 (7.86%), among respondents of Group 2 – 19 (22.09%). 15 (8.57%) of all medical practitioners mistakenly believed that it was not possible to repeat the dose at all: among respondents of Group 1 – 1 (1.12%), among respondents of Group 2 – 14 (16.28%) (Table 2).

Table 2. Knowledge about the second dose adrenaline administration

Variants of answers	Group 1		Group 2	
It is possible to repeat adrenaline administration 5 min. after the first dose.	74	83.00%	45	52.33%
It is possible to repeat adrenaline administration just after 20 min.	7	7.86%	19	22.09%
It is not possible to repeat the dose at all.	1	1.12%	14	16.28%
The second dose must be administrated just IV.	7	7.86%	8	9.30%

Source: compiled by the authors

Thus, high proportions of medical practitioners from both groups diagnose anaphylaxis in the two scenarios involving mucosal tissue demonstrating anaphylaxis with respiratory failure and anaphylactic shock. But there is a huge difference in recognising anaphylaxis with persistent gastrointestinal symptoms without involvement of the skin-mucosal tissue after exposure to a likely allergen for that patient. The respondents of Group 1 showed much better results than of Group 2. However, there was overdiagnosis by respondents of Group 1. Medical practitioners who completed ERC courses (Group 1) performance was better than those who did not practice in simulation centres (Group 2) on questions regarding dose, route, and site of adrenaline administration.

Discussion

In the study by S.N. González-Díaz *et al.* [12], 1023 respondents were scored through a questionnaire focused on the management of anaphylaxis. Results indicated that healthcare providers with over 30 years of experience, and medical students, achieved higher percentages of correct answers (50% and 39.4%, respectively). The ability to recognise, diagnose, and treat anaphylaxis, and subsequently refer patients to specialists in Allergy and Clinical Immunology, is critical for healthcare providers. The study revealed significant differences in approval rates among various speciality groups. Notably, in a post-hoc analysis, specialists in allergy and immunology had significantly higher approval rates compared to general medicine practitioners (62.9% vs. 25%; $p < 0.001$). This underscores the importance of specialised knowledge and training in the effective management of anaphylaxis.

E.J. Jares *et al.* [13] published the results of a cross-sectional study to evaluate the knowledge of doctors who have worked in allergy units from 12 Latin America countries and Spain about features of anaphylaxis by an online questionnaire designed by professional allergists. The researchers noted that epinephrine was prescribed in fewer than half of the incidents. They underscored the critical necessity to enhance the spread and application of international anaphylaxis guidelines. In the cross-sectional study conducted by D. Almarri *et al.* [14], 173 physicians completed a survey assessing their ability to identify clinical scenarios of anaphylaxis. The results showed that only 5.2% of the physicians correctly identified all three proposed clinical scenarios of anaphylaxis. Additionally, 16.8% of the respondents accurately identified two scenarios, while 51.4% managed to correctly identify only one scenario. Regarding first-line management, 42.8% recognised it correctly. However, only 24.3% knew the correct epinephrine dosage, and 24.9% identified the proper administration route. Z.A. El-Sayed *et al.* [15] indicated that Egyptian doctors' understanding and approach to anaphylaxis remain insufficient. It was shown in this survey that only 91 participants (37.6%) out of 242 respondents correctly identified all four proposed clinical scenarios of anaphylaxis. 75 respondents (31%) correctly identified the appropriate dose of epinephrine, while 119 (49.2%) correctly identified the proper administration route. Authors made a conclusion of obtained data underscored the importance of promoting

and implementing international guidelines for diagnosing and treating anaphylaxis in Egypt.

In accordance with the findings of a study by M. Serbes *et al*. [16], a notable number of participants seemed unfamiliar with the diagnostic criteria for anaphylaxis. Nearly half of the participants incorrectly diagnosed one or more scenarios. Respondents had not used recent recommendations from the EAACI regarding anaphylaxis management, especially concerning the use of epinephrine. The researcher uncovered significant gaps in the knowledge and readiness of family physicians regarding anaphylaxis. They highlighted the necessity for more effective guidance and national training programmes. These programmes should be regularly provided as part of continuous medical education to ensure physicians maintain up-to-date and comprehensive knowledge levels. In the study, it was demonstrated that the knowledge of medical practitioners does not depend on different specialities but rather depends on completed ERC courses.

Administering adrenaline stands as the central pillar of anaphylaxis management. I. Alen Coutinho *et al.* [17] noted that the staff of tertiary Hospital Emergency Department responded that the treatment for anaphylaxis preferred intramuscular route in 57.1%. Y.A. Alghasham *et al.* [18] have indicated primary healthcare providers participated in the cross-sectional study in Qassim region of Saudi Arabia demonstrated poor knowledge regarding the diagnosis and management of anaphylaxis patients: only 38.4.7% of them answered that the correct route of administration is intramuscular. While the study has shown a higher percentage 76.0% of right answers by Ukrainian healthcare staff of correct answers. There is a critical need for physicians to receive education aimed at enhancing their proficiency in promptly diagnosing and treating cases of anaphylaxis.

J.A. Pimentel-Hayashi *et al.* [19] reported that in Mexico city of 196 specialist physicians: pediatrics, internal medicine, cardiology, anesthesiology, general surgery, orthopedics, and gynecology, The study found that 96.44% of participants demonstrated correct diagnosis of an anaphylaxis case with cardiovascular, cutaneous, and respiratory symptoms. Additionally, 52% of all respondents accurately diagnosed anaphylaxis even in the absence of cutaneous symptoms. Regarding the administration route, 63.4% correctly indicated that the first dose of epinephrine should be given intramuscularly, while 50% of the participants accurately selected the appropriate dose of epinephrine. Surprisingly, only 2.6% of the participants managed to answer all 10 questions correctly. While in this study, respondents demonstrated a relatively a bit lower level of correct diagnosis (68%) for anaphylaxis scenarios involving mucosal tissue combined with hypotension. Similarly, 75.4% of all respondents correctly diagnosed anaphylaxis scenarios associated with respiratory failure. The comparison between the two groups (studies) revealed only minor differences in their responses to these particular scenarios. Overall, both studies emphasise the importance of accurate anaphylaxis diagnosis and the role of medical practitioners in understanding and effectively managing anaphylactic cases.

The study showed that Ukrainian health providers have some difficulties in the recognising anaphylaxis with persistent gastrointestinal symptoms without the involvement of skin-mucosal tissue. Similar results were obtained by J.A. Pimentel-Hayashi *et al.* [19]. Research findings of Ibrahim Irwani *et al*. indicated a tendency among doctors to overdiagnose cases of single organ involvement without hypotension as anaphylaxis. Only 42.6% out of 47 doctors of the Emergency Department were able to diagnose anaphylactic hypersensitivity in such scenarios [8]. In this study 66.0% and 68.6% respondents (in Group 1 and Group 2 respectively) overdiagnosed cases of single organ for anaphylaxis.

Studies like that of S.H. Sicherer *et al.* [20] have shown that many healthcare professionals, including doctors, lack confidence in the correct administration of adrenaline, leading to potential errors in delay in administration. It was found that 14.3% of Ukrainian respondents made a mistake answering about the timing of medicine administration.

In a longitudinal study by T. George *et al.* [21] the majority of students and staff agreed that case-based learning was a superior method of learning compared to didactic teaching. They found that case-based learning facilitated deeper understanding and promoted critical thinking more effectively than didactic teaching. Emphasising practical experiences in medical education fosters a mindset of continuous learning and adaptability to emerging medical trends. Questionnaire results from studies [7, 22] demonstrated that participants who had received anaphylaxis training and had experience managing anaphylactic cases possessed greater knowledge about the correct dosing of epinephrine.

The knowledge of Ukrainian healthcare providers regarding anaphylaxis management was found to be inadequate based on a questionnaire. Therefore, improved education and training of healthcare providers in simulation centres are essential for better anaphylaxis management. This highlights the significance of practical reinforcement of theoretical knowledge.

Conclusions

The purpose of this study was to analyse the level of knowledge of Ukrainian doctors regarding variable manifestations and first-line treatment of anaphylaxis depending on the form of training. The study reveals mixed levels of knowledge among medical practitioners regarding anaphylaxis diagnosis and treatment. While a majority of respondents relied on their university education and Ukrainian guidelines for their knowledge, there was a notable lack of familiarity with international guidelines, suggesting potential gaps in global best practices integration and mostly the half of them had have the simulation training. While respondents generally demonstrate good understanding in diagnosing anaphylactic shock with involving mucosal tissue or skin and respiratory compromise. Medical

practitioners who underwent training in simulation centres (Group 1) showed better expertise in identifying and managing anaphylaxis compared to those who did not receive such training (Group 2). Specifically, there was a notable disparity in their ability to recognise anaphylaxis with respiratory compromise and persistent gastrointestinal symptoms in the absence of skin-mucosal involvement following exposure to a likely allergen for the patient. Group 1 participants exhibited a significantly higher accuracy rate in recognising these symptoms (47.19%, 42 individuals, $p < 0.05$), whereas only 15.11% (13 individuals, $p < 0.05$) of Group 2 participants correctly identified them.

The findings highlight the significance of simulation-based training in enhancing awareness and management of anaphylaxis among Ukrainian medical practitioners. This approach aims to improve healthcare professionals' knowledge and ultimately reduce mortality from anaphylaxis. Future research should evaluate the effectiveness of simulation-based training programmes in medical education institutions across Ukraine and their potential impact on lowering anaphylaxis-related deaths.

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Conflict of Interest

The authors declare no conflict of interest.

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Порівняння ефективності методів навчання медичних працівників в Україні щодо анафілаксії

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Анотація. Кількість випадків анафілаксії зростає у всьому світі. Тому важливо, щоб медичні працівники були обізнаними щодо симптомів анафілаксії та вміли реагувати ефективно. Метою цього дослідження була оцінка рівня знань українських медичних працівників щодо анафілаксії, їхньої здатності розпізнавати різноманітні прояви анафілаксії та знань про введення адреналіну в залежності від їх форми навчання. Було проведено перехресне дослідження протягом травня 2023-червня 2023 року з участю лікарів різних спеціальностей, парамедиків, студентів медичних університетів та інтернів в Україні. Для оцінки знань клінічних критеріїв діагностики та управління анафілаксією використовувався анонімний опитувальник на основі рекомендацій European Academy of Allergy and Clinical Immunology щодо анафілаксії (оновлення 2021 року). Порівнювали дві групи: група, яка завершила курси Європейської ради реанімації у центрах симуляційного навчання (Група 1) та інша без такого досвіду (Група 2). Респонденти в основному здобували знання про анафілаксію під час університетської освіти та з українських рекомендацій, з обмеженим використанням міжнародних гайдлайнів. Приблизно половина учасників завершила курси Європейської ради реанімації, що вказує на потенційну користь практичного підсилення теоретичних знань. Група 1 (ті що завершили симуляційне навчання) демонструвала вищий відсоток розпізнавання анафілаксії у сценаріях, що комбінували симптоми зі сторони дихальної системи та шлунковокишкової ситеми без проявів зі сторони шкіри та слизових після впливу потенційного алернену та більш коректно відповідали щодо шляху введення адерналіну. Тим не менш, також спостерігалася тенденція до гіпердіагностики респондентами групи 1. Дослідження показало відмінності у діагностиці та керуванні анафілаксією серед медичних працівників, з перевагою у тих, хто завершив курси Європейської ради реанімації. Постійна освіта та тренування на основі симуляцій є важливими для зменшення смертності від анафілаксії та покращення результатів лікування

Ключові слова: анафілактична реакція; знання; лікарі; адреналін; збір даних

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The use of aqueous ozone solution in the treatment of mine blast injury with extensive soft tissue defects: A case study

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Abstract. Mine explosions frequently result in severe and complex injuries, presenting challenges in wound management and infection control. This study aimed to examine the efficacy, safety, and practical implications of ozonated water in wound care for mine explosion injuries. A 37-year-old male soldier presented with extensive lacerations of both legs sustained during a mine explosion. Following initial stabilisation, foreign bodies removal and debridement, the patient was hospitalised where he underwent surgical intervention, and negative pressure wound therapy to repair tissue damage. Microbial cultures obtained from wound samples revealed the presence of multi-drug resistant strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Antibiotic susceptibility testing indicated limited treatment options due to resistance of the isolated strains. Given the severity of the injuries and microbial colonisation, ozonated water was introduced as part of the wound care regimen. Topical application of ozonated water was initiated on admission and repeated with each dressing change. Over the course of treatment, the patient demonstrated significant improvement in wound healing, reduction in microbial burden, and resolution of infection signs. The use of ozonated water facilitated expedited wound closure and minimised the need for systemic antibiotics. This case highlights the potential of ozonated water as an effective adjunct therapy in the management of mine explosion wounds, particularly

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in cases of multi-drug resistant microbial colonisation. The findings underscore the practical value of ozonated water in improving wound care outcomes and reducing reliance on systemic antibiotics in complex trauma cases

Keywords: aqueous ozone; wound infection; antibiotic-resistant bacteria; inactivation of microorganisms; negative pressure wound therapy

Introduction

The issue of injuries caused by mines and explosive devices is highly relevant today, affecting both military personnel and civilians due to the ongoing warfare in Ukraine, which have persisted for over two years following the attack by the aggressor state. The relevance of this study is further underscored by the extensive areas of mined territory in our country, as well as regions containing unexploded ordnance.

Mine explosions are devastating events that result in a multitude of injuries, including burns, lacerations, and infections, presenting significant challenges for medical personnel tasked with providing effective wound care in the aftermath [1]. Traditional methods of wound management may be insufficient in addressing the complex nature of injuries sustained in mine explosions, particularly due to the risk of infection and delayed healing [2]. In the recent study, I. Trutyak *et al.* [3] reported that mine blast and gunshot wounds were heavily contaminated with *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Proteus vulgaris*, and *Enterococcus faecalis*. Studies conducted in conflict zones and military settings additionally have reported varying rates of multidrug resistance among microbial isolates from blast injuries. G. Loban' *et al.* [4] revealed that, since the onset of the full-scale war in Ukraine, the rates of multidrug resistance in *A. baumannii* and *K. pneumoniae* isolated from infected wounds have reached 75.0% and 80.0%, respectively. Similarly, K. Moussally *et al.* [5] reported that approximately 70% of positive cultures, primarily from patients with osteomyelitis in Gaza hospitals, exhibited multidrug resistance. The researchers found that about 65% of *Staphylococcus aureus* isolates were resistant to methicillin, while approximately 35% of *Pseudomonas aeruginosa* isolates showed resistance to ceftazidime and imipenem. Among Gram-negative isolates, 30% exhibited extended-spectrum beta-lactamases, and nearly 25% of resistant Enterobacteriaceae strains were resistant to carbapenems. Therefore, there is a critical need for innovative approaches to wound treatment that can effectively combat microbial colonisation and promote tissue regeneration.

Ozonated water, produced by dissolving gaseous ozone in water or through water electrolysis, has emerged as a promising therapeutic option for wound management due to its potent antimicrobial properties and ability to enhance tissue oxygenation and wound healing processes. According to L. Mascarenhas *et al.* [6], ozonated water can be easily distributed over surfaces or wounds using portable spray disinfection devices. Previous studies have reported the successful using ozonated water and gaseous ozone, and in some cases ozonated oil in the treatment of various types of wounds. S. Dhamnaskar *et al.* [7] observed improved healing and reduction in microbial load of diabetic foot ulcers in patients who received topical application of gaseous ozone for 30 days, 30 minutes per session comparing to the group with conventional wound management. Comparably, X. Hu *et al.* [8] successfully utilised negative pressure wound therapy (NPWT) using vacuum-assisted closure (VAC) and ozonated water flushing for treatment of diabetic foot ulcers. A. Roth *et al.* [9] demonstrated the possibility to utilise gaseous ozone to treat different infected dermal wounds. In their study on the antimicrobial activity of ozone, X. Wang *et al.* [10] discovered that gaseous ozone, and ozonated water and oil exhibited a bactericidal effect in vitro against forty strains of multidrug-resistant pathogens isolated from burn wounds. The analysis of available studies indicates that the use of ozonated water for treating mine blast injuries remains relatively unexplored. This clinical case study aimed to demonstrate the effectiveness of ozonated water, obtained by electrolysis, as an adjunctive therapy in treating complicated, infected mine blast injuries in combination with NPWT.

Materials and Methods

Patient. The patient, a 37-year-old male observed in early 2024, experienced a mine blast injury resulting in open comminuted fractures of both right and left tibia and fibula, accompanied by extensive bone and soft tissue defects with displacement of fragments. Before hospitalisation, the patient underwent shrapnel removal, wounds debridement, and external fixation. Upon hospitalisation at the Swedish-Ukrainian Medical Centre "Angelholm" on January 16, 2024, the patient complained of pain and purulent haemorrhagic discharge from the wounds of both legs. He also experienced restriction of passive and active movements, inability to bear weight on the limbs, malaise, and general weakness.

Wound management. In-hospital management of wounds included debridement, partial suturing of the wounds, installation of negative pressure wound therapy system (Confort C300, Eskişehir, Turkey), and topical application of ozonated water with each dressing change. Ozonated water was produced on demand using a custom-made pre-production prototype that utilises water electrolysis on a diamond-coated anode (Fig. 1), a recently developed technology [11].

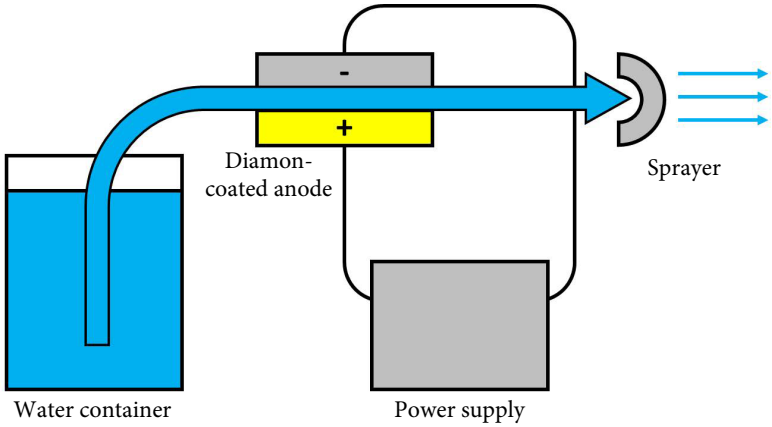

Figure 1. Principle of operation of the ozonator
Source: compiled by the authors of this study

It was sprayed over the wounds during dressing changes (Fig. 2). In cases where wounds exhibited skin defects, Vaseline gauze (Sumbow Medical Instruments Co., Ltd, Ningbo, China) was used to provide coverage and protection.

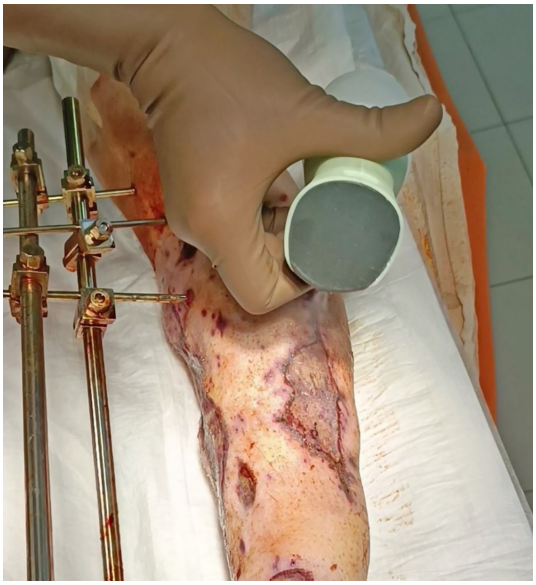

Figure 2. Application of ozonated water during the dressing change
Source: photographed by the authors of this study

Ozone concentration in water was assessed photometrically using a PoolLab 1.0 photometer (Water-i.d., Eggenstein, Germany), based on the intensity of colour change upon reaction with N,N-diethyl-p-phenylenediamine sulfate. The concentration of ozone was determined by comparing the absorption of coloured light (at wavelengths of 530 and 620 nm) by the sample to that of the untreated sample, utilising calibration data programmed into the instrument. Tablet-based reagents were used for measuring ozone concentration.

Wound cultures and antibiotic susceptibilities. The samples of wound discharge were collected on sterile cotton swabs (Jiangsu Huida Medical Instruments Co., Ltd, Yancheng, China) and transported to the laboratory using Amies transport medium. Upon the delivery the samples were inoculated onto blood agar, yolk-salt agar (Sanimed-M, LLC, Kharkiv, Ukraine), and MacConkey agar (bioMérieux, Marcy-l'Étoile, France) plates with subsequent incubation at 37°C for 24-48 hours. Initial identification of bacteria was based on their cultural and morphological properties. Gram-negative rods were identified by performing a series of tests: fermentation in Kliger Iron Agar, Simon's citrate agar (Farmaktiv, LLC, Kyiv, Ukraine), indole production, catalase production, and motility test. Gram-positive cocci were identified based on their catalase, lecithinase and coagulase (Biolik Pharma LLC, Kharkiv, Ukraine) test results.

The antibiotic susceptibility of the isolated strains was determined using the Kirby-Bauer disk diffusion method. Inocula were prepared from isolated pure cultures and diluted in sterile isotonic saline solution (0.9% NaCl). The density of the suspensions was adjusted to 0.5 McFarland units using the Biosan DEN-1 densitometer (BioSan SIA, Riga, Latvia). These suspensions were then uniformly inoculated onto Mueller-Hinton agar (Farmaktiv, LLC, Kyiv, Ukraine) plates. Antibiotic-impregnated paper disks (Farmaktiv, LLC, Kyiv, Ukraine) were placed onto the agar surface, followed by incubation at 37°C for 24 hours to allow for antibiotic diffusion and bacterial growth assessment. The diameters of the inhibition zones around the discs were measured to the nearest millimetre using a ruler. Subsequently, they were categorised as sensitive, intermediate, or resistant based on the guidelines chart provided by CLSI 2020 [12]. The antibacterials used in the test for both Gram-negative and Gram-positive strains of bacteria were ciprofloxacin (5 μg) and levofloxacin (5 μg). Gram-negative rods were tested for susceptibility to piperacillin/tazobactam (100/10 μg), ceftazidime (30 μg), cefepime (30 μg), gentamicin (10 μg), amikacin (30 μg), imipenem (10 μg), and meropenem (10 μg). Oxacillin

(1 μg), vancomycin (30 μg), and linezolid (30 μg) were used for Gram-positive bacterial isolates.

Ethics statement. The research protocol for this study was conducted in accordance with the principles outlined in Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects" [13] and the UNESCO Universal Declaration on Bioethics and Human Rights [14]. The study design and procedures were approved by the Committee on Bioethics of I. Horbachevsky Ternopil National Medical University (Protocol No. 77, April 18, 2024), and the patient provided informed consent prior to his involvement in the study. The patient's personal information was handled confidentially in accordance with ethical guidelines.

Results and Discussion

Upon hospitalisation, the patient presented with multiple wounds on both legs, exhibiting massive soft tissue defects and comminuted fractures of shin bones with purulent and haemorrhagic discharge. The fractures were stabilised with external fixators, and partial wound closure using sterile Vaseline gauze was performed (Fig. 3).

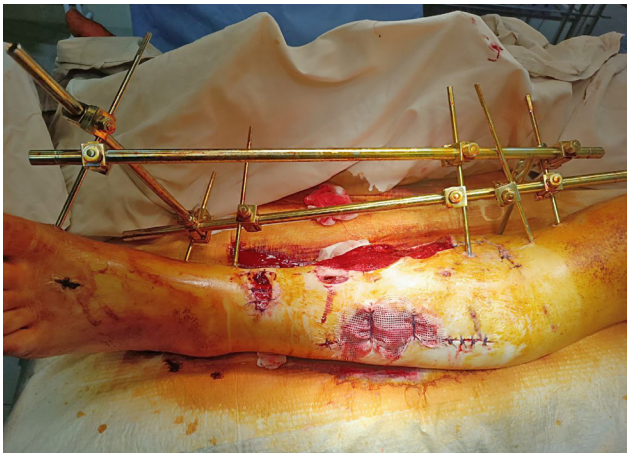

Figure 3. Angular external fixator. Partial wound closure using sterile Vaseline gauze
Source: photographed by the authors of this study

Sampling of the wound discharge revealed cultures of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The strains were resistant to almost all antibiotics tested. The *P. aeruginosa* strain was sensitive to ciprofloxacin and levofloxacin only. Similarly, *S. aureus* demonstrated susceptibility to these antibiotics as well as to vancomycin. Wound management included debridement and negative pressure wound therapy (Fig. 4 and Fig. 5). After necrectomy and the application of a negative pressure wound therapy dressing, the condition of the wounds improved and fresh granulating tissue was observed.

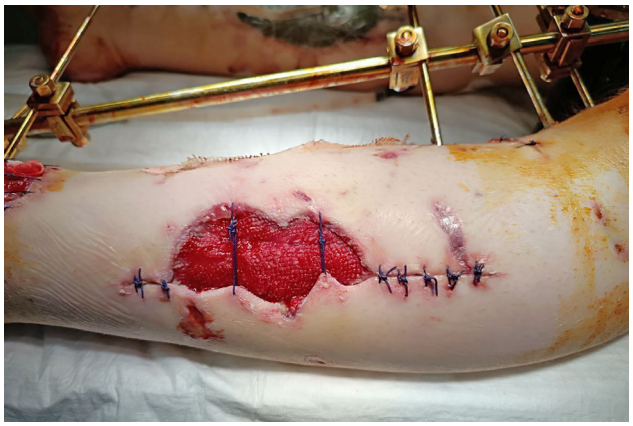

Figure 4. Granulating wound surface
Source: photographed by the authors of this study

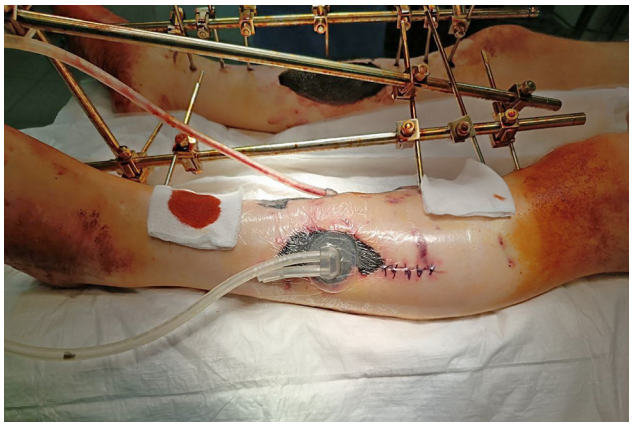

Figure 5. Negative pressure wound therapy system in situ
Source: photographed by the authors of this study

To combat antibiotic-resistant bacteria, an aqueous solution of ozone was topically applied during each wound dressing procedure. Measurements of ozone concentration revealed variations ranging from 3.10 to 4.13 mg/L. The utilisation of NPWT in combination with the application of ozonated water created favourable

conditions for wound healing. As the wounds continued to heal and granulation tissue formed, the wounds were covered with skin autografts harvested from the patient's hips to minimise the risk of rejection. The autografts provided a viable means of covering the extensive soft tissue defects, promoting further healing, and ultimately aiding in the restoration of the skin's integrity and function. (Fig. 6 and Fig. 7).

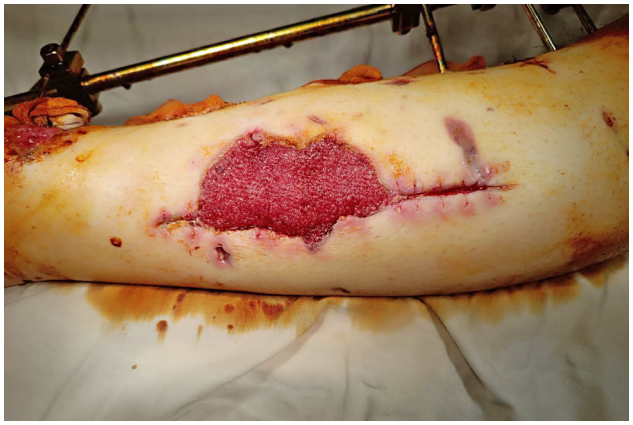

Figure 6. Granulating wound surface prepared for autologous skin grafting
Source: photographed by the authors of this study

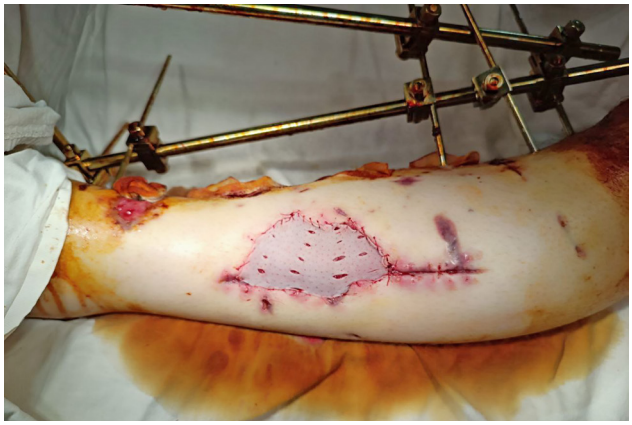

Figure 7. The wound is covered with the skin autograf
Source: photographed by the authors of this study

Following the grafting procedures, the use of ozonated water was maintained during all dressing changes and throughout the patient's hospitalisation until discharge. This continuous application played a crucial role in the successful engraftment of the grafts. The ozonated water helped keep the wound environment clean, reducing the risk of infection and promoting optimal conditions for healing.

On the fifth day after the grafting procedure, the skin flaps showed successful engraftment, which was a positive indication of the treatment's efficacy (Fig. 8). The absence of graft rejection throughout the healing process underscored the benefits of using ozonated water as part of the wound care regimen.

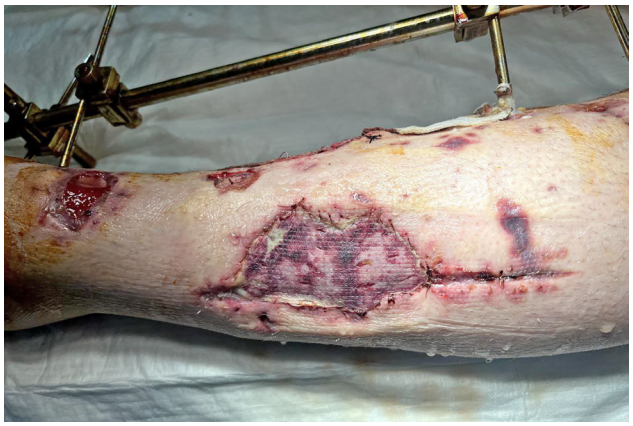

Figure 8. Day 5 after skin flap transplantation procedure
Source: photographed by the authors of this study

By day 10, complete revascularisation of the flaps was observed, indicating that the blood supply had been effectively restored and the grafts were fully integrating with the surrounding tissue (Fig. 9). The patient's wounds remained free from signs of infection, and there was a noticeable reduction in inflammation and exudate, which are common challenges in managing complex wounds. The patient's recovery was closely monitored, and the consistent use of ozonated water was maintained.

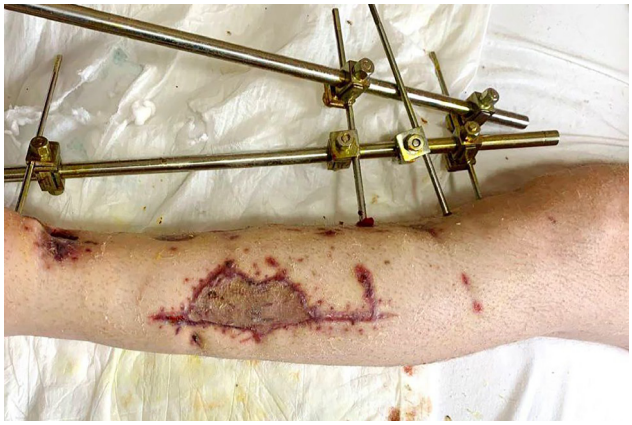

Figure 9. Day 10 after skin flap transplantation procedure
Source: photographed by the authors of this study

Throughout the course of treatment, the patient demonstrated improvement in wound healing, accompanied by the resolution of infection. The application of ozonated water for wound sanitation and treatment, in conjunction with surgical interventions and NPWT, significantly contributed to the successful management of the mine blast injuries in this patient. Upon discharge from the hospital on March 5, 2024, the patient's skin and soft

tissue wounds had healed, albeit with ongoing bone healing requiring the continued use of external fixators on both legs for stability and support.

Mine blast injuries present unique challenges in wound management due to the severity and complexity of tissue damage, including fractures or other bone injuries. Since the introduction of the first commercial vacuum-assisted closure device in 1995, subsequent research has substantiated the beneficial physiological impact of negative pressure on wound healing. Initially employed to enhance the healing process of open nonsurgical wounds by secondary intention, the clinical utility of NPWT has expanded considerably. S. Poteet *et al.* [15] reported that NPWT is now utilised not only in open surgical wounds and closed surgical incisions but also in skin graft surgery. Furthermore, advancements in device technology have led to the integration of additional functionalities and features such as instillation, antimicrobial sponges, and enhanced portability.

While NPWT creates a controlled environment that can help reduce bacterial load and promote wound healing, it may not completely eliminate the risk of infection, especially in wounds that are already contaminated or infected. In the clinical case report described, multi-drug resistant strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus* were isolated from the patient's wounds. This mirrors the findings of I. Trutyak *et al.* [3] who observed heavy contamination with *Pseudomonas aeruginosa* and enteric bacteria in mine blast and gunshot wounds. Similarly, R. Staruch & S. Hettiaratchy [16] found that *Staphylococcus aureus* and *Pseudomonas* were the causative agents for osteomyelitis in a group of 84 patients with open tibial fractures resulting from gunshot wounds and lower velocity metal fragments.

The portable custom-made ozonator considered in this study produced ozone concentrations ranging from 3.10 to 4.13 mg/L in aqueous solution. In the study by H. Li *et al.* [17], higher ozone production levels were achieved, up to 7.686 mg/L, using boron-doped diamond as the electrode material in the electrochemical ozone production process. Furthermore, they demonstrated that an ozone concentration of 4.86 mg/L in water effectively inactivated *Escherichia coli* with inoculum concentrations ranging from 1×10^3 to 3×10^9 CFU/mL. Similarly, B. Schorr *et al.* [18] reported the use of boron-doped diamond electrodes to generate reactive oxygen species for in-situ electrochemical oxidation. This method effectively eliminated *Escherichia coli*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* spores from water samples.

The successful utilisation of ozonated water in the treatment of mine explosion wounds presents a prospect for improving wound care outcomes in challenging environments. This clinical case demonstrates significant improvements in wound healing and a reduction in microbial burden following the topical application of ozonated water. These results are consistent with previous studies that have investigated the antimicrobial properties of ozone in wound management. Several studies have reported similar outcomes when using ozonated water for the treatment of various types of wounds. For instance, Hu *et al.* [8] effectively employed NPWT with VAC along with ozonated water flushing to treat diabetic foot ulcers. Their study revealed that patients who received ozonated water (10 μg/mL) administered into the VAC system twice daily experienced accelerated wound healing and lower infection rates compared to those treated with VAC alone. Similarly, A.N. Murakami *et al.* [19] investigated the use of ozonated water as an adjunct therapy for surgical site infections following pediatric cardiovascular surgery. Their results showed a significant reduction in microbial colonisation and improved wound healing outcomes in patients treated with ozonated water (0.2-0.6 mg/L) compared to standard wound care protocols. Additionally, T. Yasheng *et al.* [20] demonstrated the clinical efficacy of ozonated water (10 mg/L) lavage combined with vacuum-sealed drainage in the treatment of eighteen patients with chronic osteomyelitis in the limbs.

A. Roth *et al.* [9] described utilisation of ozone as an adjunct therapy. According to the authors while ozone therapy has drawbacks due to its toxicity at high concentrations, combining it with antibiotics could enhance treatment effectiveness without relying on high doses of ozone or antimicrobials. Ozone treatment weakens microbial cell walls, making them more susceptible to antibiotics, thus improving their effectiveness.

While the use of ozonated water did not shorten the overall treatment duration due to the inherent nature of the healing process, which requires adequate time [personal observation of O. Bilyk], it significantly improved the treatment course. The use of ozonated water reduced the dependence on antibiotics, which are often associated with various side effects. By maintaining a clean and conducive wound environment, ozonated water helped to control infection and promote healing, thereby minimising the need for systemic antibiotics. This approach not only lessened the potential adverse effects of prolonged antibiotic use but also addressed the challenge of antibiotic resistance, making the treatment more sustainable and patient-friendly.

The authors of the discussed publications utilised various forms of ozone for wound treatment. However, electrolytic ozonation, which involves generating ozone through electrolysis of water, is generally considered safer compared to conventional ozonation methods. According to E. Grignani *et al.* [21], traditional methods usually involve bubbling gaseous ozone through water, which can cause irritation to the eyes, skin, and mucous membranes upon contact. Additionally, M. Alimohammadi & M. Naderi [22] reported that gaseous ozone is toxic to humans at high concentrations and is corrosive to certain materials, such as natural rubber. As a result, electrolytic ozonation, which was utilised in this particular case, offers a safer alternative, reducing the need for expensive thermal or catalytic destructors to neutralise residual gaseous ozone.

Conclusions

This clinical case report provided compelling evidence of the successful utilisation of negative pressure wound therapy in combination with ozonated water for the effective management of mine blast injuries with extensive soft tissue defects complicated by wound infection caused by multi-drug resistant strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The combination of NPWT with ozonated water treatment provided a controlled environment that promotes wound healing by removing excess exudate, reducing edema, and enhancing blood flow to the wound bed, which accelerates tissue regeneration. Throughout the treatment course, the patient demonstrated significant improvement in wound healing, accompanied by the resolution of infection. The application of ozonated water contributed to wound sanitation and treatment. The antimicrobial action of ozonated water reduces the reliance on systemic antibiotics, which is crucial in combating antibiotic resistance and minimising the adverse effects associated with prolonged antibiotic use. Additionally, the successful integration of skin autografting further enhanced the healing process, resulting in the successful closure of soft tissue wounds. The patient was discharged from the hospital with healed skin and soft tissue wounds. He continued to wear an external fixator as his bones were still healing. This study underscores the potential of aqueous ozone solution as an adjunctive therapy in wound management, offering positive outcomes for patients with complex soft tissue injuries. It represents a promising strategy for managing complex wounds, especially those contaminated with antibiotic-resistant bacteria, ultimately leading to improved clinical outcomes and enhanced patient well-being. Moving forward, further research and clinical trials are warranted to explore the full therapeutic potential of NPWT and ozonated water in the treatment of mine blast injuries and other traumatic wounds.

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Conflict of Interest

The authors declare no conflict of interest.

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Використання водного розчину озону при лікуванні мінно-вибухової травми з великими дефектами м'яких тканин: клінічний випадок

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Анотація. Вибухи мін часто призводять до важких і складних травм, що створює проблеми з лікуванням та інфекційним контролем ран. Цей рукопис мав на меті дослідити ефективність, безпеку та практичні наслідки застосування озонованої води при лікуванні ран, отриманих від вибуху мін. Військовослужбовець, віком 37 років, отримав значні рвані рани обох ніг під час вибуху міни. Після первинної стабілізації, видалення сторонніх тіл і санації пацієнта було госпіталізовано, де йому було проведено хірургічне втручання та лікування низьким тиском для відновлення пошкоджених тканин. Мікробіологічні дослідження отриманих із зразків ран виявили наявність полірезистентних штамів *Pseudomonas aeruginosa* та *Staphylococcus aureus*. Тест на чутливість до антибіотиків показав обмежені можливості лікування через резистентність виділених штамів. Враховуючи тяжкість ушкоджень і мікробну колонізацію, озонована вода була введена в режим догляду за ранами. Місцеве застосування озонованої води було розпочато одразу при госпіталізації і повторювалося з кожною заміною пов'язки. Протягом курсу лікування пацієнт продемонстрував значне покращення загоєння ран, зменшення мікробного навантаження та зникнення ознак інфекції. Використання озонованої води сприяло швидкому загоєнню ран і мінімізувало потребу в системних антибіотиках. Цей випадок підкреслює потенціал озонованої води як ефективної допоміжної терапії при лікуванні ран від вибуху мін, особливо у випадках мікробної колонізації, стійкої до багатьох лікарських засобів. Отримані дані підкреслюють практичну цінність озонованої води для покращення результатів лікування ран і зменшення залежності від системних антибіотиків у випадках складних травм

Ключові слова: водний озон; ранова інфекція; антибіотикорезистентні бактерії; інактивація мікроорганізмів; лікування ран негативним тиском

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Microbiome of athletes: Its features and diversity: A literature review

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Abstract. The microbiome of athletes is of the greatest interest among researchers, as the microbial composition of the colon plays a key role in the absorption of nutrients, the synthesis of vitamins, and the immune response of the host body. The purpose of this review was to investigate the relationship between the gut microbiota in high-performance athletes and people with low-activity lifestyles, and the effect of these changes on the production of microbial metabolites that are associated with physical performance and athletic performance of athletes. A total of 42 research papers were analysed, including 11 specialised studies that examined the effect of intense physical activity of different types on the microbial composition of the gut and 19 studies that focused on the correlation of individual bacteria and physical performance. Gut microbial composition has been found to be associated with athletic performance and is likely to improve performance and recovery. Physical activity has been shown to increase α-diversity and microbial metabolites, such as short-chain fatty acids, compared to people who have a low-activity lifestyle. There were no significant differences in α-diversity between sports. The microbiome of athletes was characterised by a higher amount of short-chain fatty acids, which can be energy substrates during exercise. The production of short-chain fatty acids is associated with *Eubacterium rectale, Blautia* spp., *Faecalibacterium prausnitzii*. The athletes' microbiome also demonstrated the presence of *Prevotella* spp., which in athletes may correlate with performance. It has been shown that the presence of *Veillonella atypica* in athletes positively correlates with endurance. Despite the fact that the findings are contradictory, sports achievements and health of athletes specialising in various sports are associated with such types of bacteria as *Akkermansia municiphila, Faecalibacterium prausnitzii, Eubacterium rectale, Roseburia hominis*. In addition, it has been shown that there is a link between the microbial composition of the gut and enzymes that are considered key in the production of metabolites associated with the health of athletes

Keywords: microbial composition; short-chain fatty acids; *Akkermansia*; *Veillonella atypica*; sporting achievements

Introduction

The microbial composition of the human gut is currently one of the key areas of research, especially in sports medicine, as it contains a huge potential for the health and adaptation of athletes. F. Fontana *et al.* [1] found that the lifestyle of athletes, namely the amount of training combined with nutrition, modulates the gut microbiota, thereby increasing the enzymatic capabilities of the host body, which affects muscle performance. A.E. Mohr *et al.* [2] confirmed that the human gut microbiota has a great metabolic potential and contains not only thousands of taxa of various bacteria, but also microbes, viruses, archaea and, most importantly, more than three million genes, affecting the immune system,

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the absorption of nutrients, and the synthesis of certain vitamins. Moreover, the microbial composition of athletes is significantly different from the microbial composition of people who have a low-activity lifestyle. Athletes have a significantly higher α-diversity of the microbiome, and a higher number of bacteria that are associated with health. R.L. Hughes & H.D. Holscher [3] found that working with the composition of the microbiota can be a strategy for improving athletic performance, as it promotes the health of the gastrointestinal tract, and therefore, the absorption of nutrients, and is also a producer of short-chain fatty acids (SCFA), which can be energy substrates, increasing the endurance of athletes. M.T. O'Brien *et al.* [4] note that probiotics, such as *Lactobacillus* spp. and *Bifidobacterium* spp. have a protective effect against upper respiratory tract infections, which often occur in elite athletes due to the immunosuppressive effect of high-intensity physical activity. K. Mańkowska *et al.* [5] concluded that the metabolism of certain nutrients occurs with the participation of the gut microbiota. In addition, *Bifidobacterium* spp. have a protective anti-inflammatory function. To confirm the anti-inflammatory function, H.Y. Cheng *et al.* [6] found that microorganisms that are part of the human gut microbiome can interfere with the colonisation of pathogens by stimulating the production of IL-10, IL-17, and IL-22, which are antimicrobial peptides. It has been confirmed that thousands of microorganisms from various phyla, such as *Firmicutes*, *Bacteroidetes*, Actinobacteria, and *Proteobacteria*, activate the body's protective functions, preventing the development of pathogens. Moreover, M. Parizadeh & C. Arrieta [7] note that the microbiome can potentially be used in therapeutic interventions, such as faecal transplantation, pro- and prebiotic implantation. M.J.W. Furber *et al.* [8] concluded that it is the stability of the gut microbiota that can improve athletic performance. R.L. Hughes [9] also found that the gut microbiome can have a significant impact on athletic performance by producing SCFA, using lactate, and inducing enzyme activity. The predominance of bacteria in the microbial composition of the intestines of athletes, which are associated with a high level of SCFA production, can contribute to athletic achievements in endurance sports, as noted by O. Palladina [10] and G. Baldanzi *et.al.* [11]. The effect of certain microorganisms on athletic performance was investigated by J. Scheiman *et al.* [12] and it was found that *Veillonella* spp., which was found in samples of intestinal microbiome of marathon runners, significantly contributes to the endurance of athletes. The available data indicate a significant potential for the gut microbiome to improve endurance and athletic performance. So far, researchers have investigated the functions of individual microorganisms and changes in microbial composition during dietary interventions. The purpose of this study was to systematise the general and distinctive features of the microbial composition of the intestine of both high-performance athletes specialising in various sports, and in comparison with individuals who have a low-activity lifestyle.

The literature search was carried out for the keywords "gut microbiome", "athletes' microbiome", "athletes' microbiota", "athletes' gut microbiota" in such databases as Medline (PubMed), Scopus (Elsevier), Google Scholar, Plos One. Data analysis was performed in accordance with the recommendations for meta-analysis [13]. Only randomised controlled trials, meta-analyses, and systematic reviews published between 2017 and 2024 were considered. 42 research papers were found. The criteria for inclusion in the analysis of the first division were studies on people (n>4 in each group) who were professionally engaged in sports. The second subsection included scientific sources that considered the effects of individual bacteria on physical performance.

The Difference Between the Microbiome of Athletes and People who do not Exercise

The results of studies that were conducted between 2017 and 2024 were compared (and also included a paper published in 2014, as the results became the basis for future research), the conclusions are set out in Table 1. It has been shown that there are significant differences between the microbiota of athletes and the microbiota of people who have a physically inactive lifestyle [14-16]. A greater α-diversity of microbiota was recorded in athletes, while the level of microorganisms of the *Bacteroidetes* phylum was reduced. Both athletes and people who have an active lifestyle are dominated by bacteria such as *Akkermansiaceae* and *Faecalibacterium* spp. [10, 17].

Table 1. Composition of the gut microbiota of athletes depending on the sport or in comparison with non-athletes

	Subjects	Year	Method	Features of the microbiota	Source
1	Various sports with aerobic and anaerobic loads (in particular, cyclists and rugby players) (n = 186)	2023	Shotgun metagenomic sequencing	*Eubacterium rectale, Blautia* spp., *Faecalibacterium prausnitzii*, etc. unclassified species of *Faecalibacterium*, *Ruminococcus bromii*, unclassified species of *Eubacterium* and *Ruminococcus*.	1
2	Irish athletes (n = 27) in 16 different sports	2020	Shotgun metagenomic sequencing	No differences in microbial composition were established depending on the sport. The samples were dominated by species of one or a combination of five species: *Gordonibacter massiliensis, Eubacterium rectale, Faecalibacterium prausnitzii, Bacteroides vulgatus*, and *Polynucleobacter necessarius*. Microbiota of athletes who specialised in dynamic sports (such as field hockey) was dominated by *Bifidobacterium animalis*, *Lactobacillus acidophilus, Prevotella intermedia*, and *F. prausnitzii*. Microbiota of athletes in sports that combine both dynamism and static (such as rowing) had higher amounts of *Bacteroides caccae.*	18

Continued Table 1

	Subjects	Year	Method	Features of the microbiota	Source
3	Male non-athletes (control group n = 10), bodybuilders (n = 15), runners (n = 15)	2019	Amplification of the 16S rRNA gene in the V3 and V4 regions	α and β diversity did not differ in different types of sports. Bodybuilders had the highest levels of *Faecalibacterium* spp., *Sutterella* spp., *Clostridium* spp., *Haemophilus* spp., and *Eisenbergiella* spp.	19
4	Athletes in endurance sports (n = 15), athletes in power sports (n = 16), control group (n = 21)	2024	Amplification of the 16S rRNA gene	No significant differences were found in α- and β- diversity between groups. In endurance athletes, the enterotype with *Bacteroide* spp. *s* dominated, while in strength athletes, the enterotype with *Prevotella* spp. prevailed. Positive correlations were found between SCFA producers (*Blautia wexlerae, Eubacterium rectale*, and *Intestinimonas timonensis*) and maximum power during the Wingale anaerobic test.	20
5	Rugby players (n = 40) and non-athletes with a BMI within the normal range (n = 46)	2014	Amplification of the 16S rRNA gene in the V4 region	Higher proportion of *Akkermansia* spp. compared to the control group.	21
6	Rugby players (n = 40) and non-athletes with a BMI within the normal range (n = 46)	2018	Shotgun metagenomic sequencing	Greater diversity of the athletes' microbiota compared to the control group. Higher number of SCFA.	22
7	Professional cyclists (n = 22) and amateur cyclists (n = 11)	2017	Shotgun metagenomic sequencing and RNA sequencing	No correlations were found between taxonomic groups. Higher relative abundance of *Prevotella* spp., depending on the number of training sessions (> 11 hours/week). Increased number of *Methanobrevibacter smithii* transcripts in professional athletes and low number of Bacteroides. 30 out of 33 athletes had *Akkermansia* spp.	23
8	Marathon athletes (n = 15) and subjects who have a sedentary lifestyle (n = 10)	2019	16S rDNA sequencing	Increase in the relative number of representatives of the genus *Veillonella* in athletes after the marathon.	12
9	Martial artists (n=31)	2019	Amplification of the 16S rRNA gene in the V3 and V4 regions	*Parabacteroides* spp., *Phascolarctobacterium* spp., *Oscillibacter* spp., and *Bilophila* spp. prevailed in higher-level athletes. *Megasphaera* spp. prevailed in lower-level athletes.	24
10	Marathon runners (n=14), skiers (n=11), control group (n=46)	2020	Amplification of the 16S rRNA gene	High ratio of *Prevotella* spp. to *Bacteroides* spp.	25
11	Triathletes (n=4), control group – healthy men with a BMI within the normal range (n=4)	2023	Microbial DNA sequencing	More α-diversity in athletes, and reduced levels of *Bacteroidetes*, increased levels of *Akkermansiaceae, Faecalibacterium* spp.	10

Source: compiled by the authors based on the data shown in the Table

Establishing a causal relationship between sports and the microbiome of athletes is quite problematic. The results can be influenced by environmental factors, dietary interventions, antibiotic use, differences in the intensity of physical training and its duration, methods of preparation and logistics of samples, databases, and bioinformatics methods [26, 27].

S.F. Clarke *et al.* [21] studied rugby athletes and found that they had a higher proportion of representatives of *Akkermansia* spp., compared to the control groups. The researchers note that dietary interventions, especially protein intake, may have affected an increase in the diversity of gut microbial composition. W. Barton *et al.* [22] continued their research by examining the samples using the shotgun metagenomic sequencing method. Their task was to understand the metabolic capacity and taxonomic composition. As a result, when studying the differences between the group of athletes and the control group, people who have an inactive lifestyle, more differences were demonstrated at the metabolic and metagenomic levels compared to the level of the gut microbiota. In addition, athletes were found to have higher amounts of short-chain fatty acids, particularly butyrate, propionate, and acetate, and increased ability of carbohydrate metabolism, protein synthesis, and other metabolic pathways compared to the control group.

L.M. Petersen *et al.* [23] analysed the difference in microbiota depending on the level of athletes. Although no significant correlations were found between taxonomic groups, however, the amount of exercise positively correlated with the abundance of *Prevotella* spp. In addition, professional cyclists had an increased number of *Methanobrevibacter smithii* transcripts compared to amateur cyclists and the low number of Bacteroides. In 30 of the 33 athletes *Akkermansia* spp. were isolated, which is usually associated with a healthier metabolic profile [28].

C.M. O'Donovan *et al.* [18], when studying differences in the quantitative composition of health-related bacteria

among high-performance athletes specialising in various sports, found that there were no differences in microbial composition, depending on the sport. However, such spesies as *Gordonibacter massiliensis, Eubacterium rectale, Faecalibacterium prausnitzii, Bacteroides vulgatus, Polynucleobacter necessarius* were observed in samples. The species were either present in relatively equal quantities or exhibited a predominance of one over the others. It was also shown that athletes who specialised in more dynamic sports (such as field hockey) were higher numbers of likely to have *Bifidobacterium animalis, Lactobacillus acidophilus, Prevotella intermedia and F. prausnitzii,* while athletes in sports that combine both dynamism and static (such as rowing) correlated with higher numbers of *Bacteroides caccae.* In a study involving bodybuilders and athletes-runners, it was shown that α and β diversity did not differ in different types of sports. Bodybuilders had the highest levels of *Faecalibacterium* spp., *Sutterella* spp., *Clostridium* spp., *Haemophilus* spp. *and Eisenbergiella* spp. [19].

R. Liang *et al.* [24] observed athletes specialising in martial arts at various competitive levels. Athletes of the highest competitive level had the prevalence of *Parabacteroides* spp., *Phascolarctobacterium* spp., *Oscillibacter* spp. and *Bilophila* spp., while the second group of athletes belonging to a lower competitive level had a higher number of *Megasphaera* spp. Separately, the training volume was monitored, i.e., the amount of time that participants trained during the average week. Increased training volume was positively correlated with the abundance of *Parabacteroides* spp.

M. Kulecka *et al.* [25] found that marathon runners and healthy non-athletes (control group) differed in 20 bacterial taxa, while the difference between skiers and the control group was 5 taxa. Both groups of athletes had low levels of the main genus of gut microbiota, *Bacteroidetes*, and a larger number of *Prevotella* spp. In addition, a greater diversity of the microbiome was inherent in athletes-skiers, compared with the control group, people who have an inactive lifestyle. Microbial composition was also found to correlate with the participants' diet. Folic acid intake increased the amount of *Christensenellaceae*, fibre positively correlated with *Agathobacter* spp., sucrose reduced the amount of *Prevotella* spp., and polyunsaturated fatty acids were inversely correlated with *Phascolarctobacterium* spp.

Published in 2024, the results of a study of the microbiome of athletes did not reveal significant differences in α and β-differences between the control group, the strength sports group, and the endurance sports group. Enterotype *Bacteroides* was predominant in athletes specialising in endurance sports, while *Prevotella* enterotype was common in athletes in strength sports. *Blautia wexlerae, Eubacterium rectale* and *Intestinimonas timonensis*, the main producers of SCFA, positively correlated with maximum power during the Wingale anaerobic test. It is important to note that SCFA can be used as an additional energy substrate, which is necessary for endurance sports [20].

It was shown that the microbial composition of the intestines of athletes is significantly more abundant and higher in α-diversity compared to subjects who have and a physically inactive, although there is no difference in diversity of intestinal bacteria, depending in the sport. The microbiome of athletes is dominated by producers of short-chain fatty acids, such as *Blautia wexlerae*, *Eubacterium rectale* and *Intestinimonas timonensis* related to health, and with greater energy potential. In addition, *Akkermansiaceae* and *Faecalibacterium* spp. significantly predominate in athletes and the number of *Bacteroidetes* is reduced, which signals the possibility of adaptation and endurance.

Potential Mechanisms of Microbiome Influence on Physical Performance

It has been found that the microbial composition of the intestines can potentially affect endurance, adaptability, and athletic performance. As already mentioned, athletes have predominantly *Akkermansia municiphila*, *Faecalibacterium prausnitzii, Eubacterium rectale, Roseburia hominis*, which are associated with sports results [15]. The presence of *Prevotella* spp. signals better results in power sports, while the predominance of SCFA producers *Blautia wexlerae, Eubacterium rectale* and *Intestinimonas timonensis* can improve results in endurance sports [20].

K. Gross *et al.* [29] demonstrated that a microbial-mediated mechanism can affect the ability to exercise endurance. Analysis of samples provided by marathon athletes 5 days before and after the marathon showed a significant increase in the relative numbers of *Veillonella* spp. for athletes after the marathon. *Veillonella* is a Gram-negative bacterium that uses lactate as its main source of energy, given that marathon running is characterised by a high level of lactate production by skeletal muscles. Later, a human strain of *Veillonella atypica* was isolated and it was demonstrated in an experiment on mice that this strain increased the time on the treadmill while the mice performed tasks. Further experiments have shown that lactate can enter the intestines, where it is catabolised by *Veillonella atypica* and it is converted to short-chain fatty acid propionate [12].

Metagenomic samples of athletes (shotgun sequencing) specialising in various sports were analysed and it was found that athletes have a greater variety of species of microbes that produce short-chain fatty acids, compared with the control group of subjects who have a sedentary lifestyle. Indeed, evidence suggests that physical activity increases α-diversity and microbial metabolites, such as short-chain fatty acids [14, 30, 31]. The latter are energy substrates during endurance exercise [32, 33]. This includes *Eubacterium rectale, Blautia* spp., *Faecalibacterium prausnitzii* and other unclassified species *Faecalibacterium*, *Ruminococcus bromii*. These taxa of bacteria are considered to be the "core" of producers of short-chain fatty acids. The athletes' microbiome also demonstrated the presence of *Prevotella* spp., or rather a dominant species *Prevotella copri.* In the control group, the presence of *Prevotella* spp. although was associated with the presence of inflammatory diseases in non-athletes, its presence may be correlated with performance in athletes [1].

It has been found that the microbial composition of the gut can affect the health of the host. The microbiota of the gastrointestinal tract promotes the absorption of nutrients and the synthesis of vitamins, which is extremely important, since physical activity can increase the rate of energy metabolism in skeletal muscles from 20 to 100 times [34-36]. The connection between the microbe and the modulation of inflammation and the body's immune response is also known [37-39]. Moreover, the results of recent studies confirm the correlation between the athletic performance of athletes and the composition of their microbiota [10-12].

It was found that despite the great scientific interest in the topic of the athlete microbiome, the available scientific literature mainly focuses on a limited range of bacteria that are well studied. Research focuses on the products of key metabolites, in particular, short-chain fatty acids, lactate, and the involvement of association with dietary interventions. Until now, little is known about the physiological mechanisms involved in resident bacteria that are modulated by physical activity and their impact on the host in terms of physical performance and overall health. Only one paper has investigated the relationship between enzymes associated with the synthesis of metabolites that promote host health and the composition of the microbiome inherent in athletes. The researchers identified a number of enzymes associated with antibacterial properties, vitamin synthesis, reduced risk of cardiovascular diseases, energy metabolism, and antioxidant properties (Table 2). These findings confirm that athletes' microbiota can potentially encode a much wider range of microbial metabolites than is currently known, with important effects on health and physical performance.

Table 2. Cluster of enzymes that correlates with the microbiome inherent in athletes

Enzyme	Final product function
Spermidine synthase	Reducing the risk of CVD mortality
Porphobilinogen synthase	Heme synthesis
Mycothiol synthase	Antibacterial and antitumor properties
Hydrogenobyrinic acid a,c-diamide synthase	Biosynthesis of coenzyme B12 (cobalamin)
Cystathionine gamma synthase	Energy metabolism, muscle function, antioxidant
Glutamate synthase (NADPH), Glutaminyl-tRNA synthase	Excitatory neurotransmitter, homocysteine balance

Source: [1]

Studies of the microbiome of athletes have certain limitations that need to be considered in the further studies. The individual microbial composition of the intestine is affected by dietary interventions, the volume and intensity of training, changing the location, taking antibiotics and other medications. The results are also influenced by the samples and their number, and the methodology chosen by researchers. In addition, it is necessary to consider the possibilities of individual variations in the microbiome of athletes, for example, with the help of probiotics. Probiotics have been shown to promote the absorption of branched-chain amino acids, and increase the amount of glycogen in the muscles and liver [40]. The gut microbiome has also been shown to accelerate erythropoiesis, so researchers have suggested that probiotic supplementation may improve athletic performance in sports with aerobic muscle energy [41]. However, the results of the study do not confirm such conclusions, which most likely indicates the compensatory mechanisms of the athlete's body [42]. Studies show that physical activity increases the diversity of the microbiome and the number of metabolites important for physical performance. However, there are still many unknown aspects of the microbiota's impact on athletes' health and physical performance.

Conclusions

The authors set out to analyse the available studies published in the period 2017-2024, and to make a comparative analysis of the microbial composition of the intestines of athletes specialising in various sports, people having a low-activity lifestyle, and to establish how changes in the microbiome due to physical exertion can affect athletic performance, adaptive capabilities, and endurance of athletes.

The microbiota of athletes does not have a significant difference in α-diversity between sports, but it differs from the microbiota of non-athletes. The *Akkermansiaceae* and *Faecalibacterium* spp. significantly predominate in athletes. Reduced numbers of *Bacteroidetes* have been shown in athletes, signalling adaptability and endurance capabilities, although other studies have found that endurance athletes had a dominant *Bacteroides* enterotype, and strength athletes had a dominant *Prevotella* enterotype, which may correlate with performance in athletes. Elite rugby players were found to have higher amounts of short-chain fatty acids, such as butyrate, propionate, and acetate, and increased ability to metabolise carbohydrates, protein synthesis, and other metabolic pathways compared to the control group. Short-chain fatty acids can be used as energy substrates and are important in endurance sports. They are usually associated with *Eubacterium rectale, Blautia* spp., *Faecalibacterium prausnitzii*. In addition, in some studies, the microbiome of athletes showed a higher proportion of *Akkermansia* spp., which is usually associated with a healthy metabolic profile. The amount of exercise was positively correlated with a larger numbers of *Prevotella* spp., which in athletes may correlate with performance, since this bacterium uses lactate as an energy source. An increase in the numbers of *Veillonella* spp. in athletes after a marathon was shown. This bacterium is associated with the highest endurance

of athletes, as it uses lactate as an energy source. A positive correlation was also found between the production of enzymes associated with the synthesis of metabolites that contribute to host health and the composition of the microbiome inherent in athletes. These enzymes are associated with antibacterial properties, vitamin synthesis, reduced risk of cardiovascular diseases, energy metabolism, and antioxidant properties.

The practical significance of this study is the identification of specific bacteria inherent in high-performance athletes to develop various interventions that will correct the intestinal microbial composition of athletes to enhance adaptive capabilities, endurance and improve athletic performance. Further research with a larger sample of athletes is required, considering other factors that may influence the outcome, such as weight changes, diet, and training periods.

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None.

Conflict of Interest

The authors declare no conflict of interest.

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Мікробіом спортсменів: його особливості і різноманіття: огляд літератури

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Анотація. Мікробіом спортсменів викликає найбільший інтерес серед дослідників, так як мікробний склад товстого кишківника відіграє ключову роль у засвоєнні нутрієнтів, синтезі вітамінів та імунній відповіді організму хазяїна. Метою даного огляду було вивчити зв'язок між кишковою мікробіотою у спортсменів вищих досягнень та людей із малоактивним способом життя, а також вплив цих змін на продукцію мікробних метаболітів, які пов›язані з фізичною працездатністю та спортивними досягненнями атлетів. Було проаналізовано 42 дослідження, з яких 11 спеціалізованих досліджень, які вивчали вплив інтенсивних фізичних навантажень різних видів на мікробний склад кишківника та 19 наукових робіт, які фокусувались на кореляції окремих бактерій та фізичної працездатності. Було виявлено, що мікробний склад кишківника пов'язаний зі спортивними результатами, і, вірогідно, може підвищувати продуктивність та відновлення. Було показано, що фізичні навантаження збільшують α-різноманітність і мікробні метаболіти, такі як коротколанцюгові жирні кислоти, порівняно з людьми, які ведуть малоактивний спосіб життя. Не було виявлено суттєвої відмінності у α-різноманітності між видами спорту. Мікробіом атлетів відрізнявся вищою кількістю коротколанцюгових жирних кислот, які можуть бути енергетичними субстратами при фізичних навантаженнях. Продукцію коротколанцюгових жирних кислот пов›язують з *Eubacterium rectale, Blautia* spp., *Faecalibacterium prausnitzii*. Мікробіом атлетів також продемонстрував наявність *Prevotella* spp., яка у спортсменів може корелювати з продуктивністю. Було показано, що наявна у атлетів *Veillonella atypica* позитивно корелює з витривалістю. Незважаючи на те, що результати досліджень є суперечливими, спортивні досягнення та здоров´я атлетів, що спеціалізуються у різних видах спорту, пов´язують з таким видами бактерій як *Akkermansia muciniphila, Faecalibacterium prausnitzii, Eubacterium rectale, Roseburia hominis*. Окрім того, було показано, що існує зв´язок між мікробним складом кишківника та ферментами, які вважаються ключовими у продукції метаболітів, що пов›язані із здоров'ям спортсменів

Ключові слова: мікробний склад; коротколанцюгові жирні кислоти; *Akkermansia*; *Veillonella atypica*; спортивні досягнення

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Pharmaceutical quality assurance methods comparison in Ukraine and the world: A literature review

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Abstract. The study of quality assurance methods for medicinal products in Ukraine and other countries is relevant to the improvement and development of the pharmaceutical industry. The study aimed to compare the quality systems for the supply of medicines in Ukraine and abroad. The structural and logical analysis methods, as well as bibliosemantic and analytical-synthetic methods, were used in the study. The comparison was conducted by analysing documents issued in foreign countries by regulatory authorities, such as the Food and Drug Administration in the United States of America or the European Medicines Agency in Europe, which is substantial in ensuring the quality of medicines. In Ukraine, the State Service of Ukraine on Medicines and Drugs Control is a regulatory body. The analysis revealed several differences in the quality control systems for medicines in Ukraine and abroad. Good Manufacturing Practice standards are universally recognised for quality assurance in pharmaceutical production. Compliance with the standards is mandatory in Ukraine and abroad. Regular testing of medicines in authorised laboratories is crucial to ensure their safety and effectiveness. Ukraine, similarly to other countries, conducts quality control testing. Monitoring and reporting of adverse drug reactions after the sale is essential to identify and address safety issues. Although pharmacovigilance systems exist in Ukraine and abroad, there may be differences in terms of reporting requirements, infrastructure and resources allocated to pharmacovigilance activities. Scheduled inspections of production facilities and distribution channels are carried out to verify compliance with the rules. Educating healthcare professionals and the public about the importance of ensuring the quality of medicines contributes to informed decision-making. The analysis of the quality control of medicinal products suggests that the fundamental methods of quality assurance are similar around the world, while differences in the regulatory framework, resources, infrastructure and implementation may affect the efficiency and effectiveness of quality assurance measures between Ukraine and other countries. Collaboration, compliance with international standards and continuous improvement are essential to promote quality assurance practices in Ukraine and abroad

Keywords: regulatory authorities in medicine; medicinal products; good manufacturing practice; pharmacovigilance; side effects

Introduction

Ensuring the quality of medicines is an important component of Ukraine's healthcare policy. Modern approaches to quality assurance are based on a concept that ensures the proper circulation of medicines. The provision of information to physicians and patients about medicinal products begins at the stage of their pharmaceutical development and is based on laboratory and clinical trials, control of medicinal product production, quality, appropriate storage and sales conditions. In other words, a concept has been developed according to which quality and safety guarantees are provided at all stages of the life cycle of a medicinal product. All measures aimed at implementing the concept of ensuring the proper quality of medicines, following the recommendations of the World Health Organization

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(WHO), are aimed at meeting the needs of patients [1]. According to the concept of development of the pharmaceutical industry in Ukraine for 2011-2023, the main task is to ensure the quality, safety and efficacy of medicines, thereby achieving the goal of improving public health and increasing life expectancy [2].

At the present stage, the Ukrainian pharmaceutical market is actively developing, raising questions about the proper quality assurance of medicines and the prevention of drug fraud. To improve the Ukrainian quality system for medicines, a thorough analysis of the experience of different countries is needed. Analysis and comparison of the experience of other countries will also help to provide the population with quality medicines and improve the existing system of regulation and control of the country's pharmaceutical market. In terms of drug quality standards, Ukraine is still trying to achieve a quality of medicines comparable to many developed countries, which affects the ability to provide the population with quality and safe medicines [3]. Despite the constant development of the pharmaceutical industry in Ukraine, there have been few studies comparing the structure of the pharmaceutical industry in Ukraine with other countries. Such studies would help to improve and enhance the efficiency of pharmacy professionals and help to achieve the main goal of providing patients with the highest quality, most effective and safe medicines.

S.H. Ubohov *et al.* [4] noted that the safety of medicines is a priority for healthcare systems in all developed countries, and Ukraine is no exception. No medicine in the world is 100% safe for the human body. S. Sarana & P. Noha [5] demonstrated that the main goal of all structures of the pharmaceutical industry in Ukraine is to ensure public access to quality and safe medicines. H. Buschmann *et al.* [6] emphasise that global information exchange between countries on adverse events enhances the safety of medicines in countries and can lead to timely policy decisions to protect patients in the event of adverse drug events. N. Zarivna [7] highlighted the main issues related to the development of draft quality control methods for finished medicines depending on the type of dosage form when studying the discipline "Standardisation of Medicines" by higher education students. The article analyses the main issues that students may have when developing appropriate specifications for medicinal products. This study concludes that knowing its requirements for quality indicators of medicinal products, analysis methods, a higher education student will learn not only to develop drafts of appropriate methods for quality control of medicinal products but will also be able to apply them in their practical activities to confirm the quality assessment of medicinal products. The legislation of Ukraine and the EU in the field of quality assurance of medicinal products was compared by L. Yurkovska *et al.* [8]. Ukrainian scientists have found that the EU and Ukrainian pharmaceutical legislation on quality assurance of medicinal products have common features, including the number and diversity of existing regulations, which create contradictions between them and the possibility of regulatory gaps. The main trend in the development of this legislation in the EU and Ukraine is the inconsistency of legislative acts and the lack of significant legislative efforts to codify them.

In other words, all the analysed studies were aimed at studying the methods of quality assurance of medicinal products and ways to improve them, but the works of other authors almost do not compare the structures of quality assurance of medicinal products with similar methods and structures in other countries. Given the above, the study aims: to compare the current state and trends in the development of quality assurance of medicinal products in Ukraine and abroad by analysing regulatory sources for pharmaceutical products, and EU and Ukrainian legislation that defines the basis for quality assurance of medicinal products. The study analysed literature sources for 2018-2024 related to the methods of ensuring the quality of medicines in Ukraine, the EU and the USA, and examined the main documents and laws that ensure the quality of medicines in these countries. The literature search was conducted in scientometric databases (Scopus, Web of Science, PubMed, EMBASE) and on the website of the Ministry of Health of Ukraine. The following keywords were used to search the literature: "regulatory authorities in medicine", "medicinal products", "good manufacturing practice", "pharmacovigilance", "adverse events".

The methods of structural and logical analysis were used to identify scientific data from the analysed literature sources according to a certain system, classify them, and establish interconnections and relationships between them. The bibliosemantic method was used to determine the state of the art of studying the safety and quality of medicines and ways to improve them by analysing the results and conclusions of previous studies based on scientific sources and modern electronic resources. The analytical and synthetic methods provided a comparison of various elementary theoretical and causal relationships as a result of treatment and also met the requirements for studying the processes of side effects as a holistic research object interconnected with a complex of influencing factors and as a fragmentary element requiring a detailed analysis of each of the individual characteristics.

Key Aspects of Drug Quality Control in Ukraine

Quality assurance of medicinal products in Ukraine includes the following methods. One of the key links in ensuring the quality of medicinal products is regulatory supervision, which ensures the implementation and control of compliance with the requirements of the legislation by state authorities, such as the State Service of Ukraine on Medicines and Drugs Control (SMDC). The State Administration on Medicines is the main body responsible for regulating the circulation of medicines in Ukraine. This includes registration of medicinal products, issuance of licences for their manufacture and distribution, as well as control over their quality and safety, including inspections of production facilities and warehouses, sampling and analysis, and verification of compliance with the

requirements of Good Manufacturing Practice (GMP). The State Administration on Medicinal Products (hereinafter SAMP) monitors and analyses adverse reactions to medicinal products and maintains a pharmacovigilance system to ensure the safety and efficacy of medicinal products and is responsible for controlling the circulation of narcotic drugs, psychotropic substances and precursors in Ukraine, including licensing, registration, inspections and control over the legalisation of revenues from their circulation [9]. In general, the SAMP aims to ensure the safety, efficacy and availability of medicines and control drug trafficking in Ukraine following the requirements of national and international legislation [10].

Similar to European countries, drug manufacturers in Ukraine must comply with GMP standards to ensure product quality. The main provisions of the GMP were adopted as the basis for the licensing conditions for the manufacture of medicines in 2011 [11]. At that time, not all drug manufacturers in Ukraine were able to ensure that their products met the requirements of the GMP. The introduction of new licensing conditions resulted in a 20% reduction in the number of drug manufacturers [12]. In 2014, 110 companies in the Ukrainian pharmaceutical market had a production licence, which did not take a wait-and-see attitude but actively implemented innovative technologies, new drugs, quality assurance systems, principles and rules of the GMP [13]. Pharmaceutical companies that met GMP requirements were certified for compliance with GMP requirements at the national level and by inspectors from other countries. The pharmaceutical industry in Ukraine is constantly monitoring the quality of its products through regular quality checks in designated laboratories to verify compliance with standards and specifications.

Monitoring and reporting of adverse drug reactions to identify and resolve possible quality issues is one of the main functions of pharmacovigilance. The state pharmacovigilance system in Ukraine was established in 1996. Currently, the system is represented by the following links: central (the State Expert Centre of the Ministry of Health of Ukraine); regional (regional branches of the Centre in all regions of Ukraine); local (pharmacovigilance in healthcare facilities) [14]. In addition to the state pharmacovigilance system, everyone who applies for registration of a medicinal product in Ukraine must establish and maintain a pharmacovigilance system with an agreed quality system. If the above condition is not met, the medicinal product will be prohibited for use in Ukraine. After all, the main task of pharmacovigilance – improving patient treatment and safety during the use of medicines – is only possible if reliable and objective information on safety (the occurrence of adverse drug reactions) and efficacy of medicines has been previously provided. Pharmacovigilance departments receive the necessary information on the side effects of medicines and the achievement of treatment effects from the holders of marketing authorisations, doctors participating in clinical trials and doctors directly treating patients. Ukrainian specialists have extensive experience in using passive and active methods of collecting information on adverse effects during the use of medicines [15].

Pharmacovigilance is of particular importance for the implementation of state healthcare programmes for the treatment of serious diseases, socially dangerous diseases and the prevention of controlled infections. Pharmacovigilance authorities ensure that manufacturing facilities and supply chains are regularly inspected to verify compliance with production standards and requirements [16]. Manufacturers that meet the quality standards are issued certificates to confirm their compliance. Cooperating with international organisations and regulatory bodies to share information and agree on best practices in quality assurance. These methods help ensure a high level of quality of medicines available in Ukraine and ensure their safety and efficacy for patients [17, 18].

According to WHO estimates, the prevalence of counterfeit medicines in developed countries is about 10%, and in third-world countries, it is almost half of all medicines [19]. In Ukraine, from 0.3% to 10% of counterfeit medicines are sold in different versions. In addition, while in 2013, 99 batches and 68 names were detected and banned in Ukraine, in 2014, in the first eight months alone, 309 batches and 69 names of substandard drugs were detected and banned. And this is a calculation of only the number of names and batches of drugs, without indicating the total number and cost of such counterfeit drugs, as it is almost impossible to calculate them [20]. Counterfeit medicines pose a high threat to the lives and health of Ukrainian citizens. Following the publication of such information about the increase in the number of counterfeit medicines, temporary control commissions were set up in many cities of Ukraine to study the situation around the circulation of counterfeit medicines [21].

The main task of the commissions is to check the distribution of medicines through the wholesale and retail network. In 2019-2024, due to the improvement of the regulatory framework responsible for the quality of medicines and the intensification of inspections by the State Administration of Ukraine on Medicines and Drugs Control, the likelihood of detecting counterfeit medicines has significantly decreased [22]. Due to the continuous improvement of pharmacological legislation, control over the counterfeiting of medicines in Ukraine has been strengthened. Thus, in August 2023, during a special operation in Kyiv and Kharkiv regions, law enforcement officers successfully identified and eliminated a channel for the illegal transportation of counterfeit oncology medicines to Ukraine. Most of the counterfeit medicines were manufactured in several countries in the Middle East [23].

The opening of a laboratory for quality analysis of medicines and drugs in the Kyiv region is a significant achievement. In this institution, specialists will conduct tests according to the highest European standards to identify low-quality medicines. According to the Ministry of Health of Ukraine, the laboratory is unique for Ukraine, and its qualifications have already been confirmed by the

World Health Organisation. The new institution will become a full member of the international network of official quality control laboratories in Europe. The latest laboratory equipment can perform complex analyses using a spectrometer, which can be used to determine the content of metals and elements in medicinal products; heavy metals and elements in medicinal plant materials; microbiological purity of medicinal products, quantification of antibiotics, vitamins, and preservatives. These changes will significantly reduce the likelihood of counterfeit medicines appearing on the pharmaceutical market [24].

To improve the pharmaceutical industry in Ukraine, the approach to professional development of pharmaceutical employees is constantly being improved. Thus, starting from 1 January 2024, all categories of specialists in the medical and pharmaceutical fields, without exception, must undergo continuing professional development (CPD). One of the main documents regulating CPD in the field of medicine is the Resolution of the Cabinet of Ministers of Ukraine No. 725 "On Approval of the Regulation on the System of Continuous Professional Development of Medical and Pharmaceutical Workers" [25]. According to the new rules, pharmaceutical employees must attend professional conferences and congresses annually, listen to webinars and take tests based on conference materials. Such an approach will significantly improve the knowledge of pharmaceutical workers and the level of patient care.

Order of the Ministry of Health of Ukraine No. 446 "Some Issues of Continuous Professional Development of Doctors" [26], Order of the Ministry of Health of Ukraine No. 742 "On the Certification of Junior Specialists with Medical Education" [27], Order of the Ministry of Health of Ukraine No. 2136 "On Approval of the List of Cycles of Specialisation and Thematic Improvement in Medical and Pharmaceutical (Pharmacy) Specialties" [28] are also in effect. However, the rules for calculating CPD points for pharmacists have not yet been finalised at the legislative level. Ukrainian scientists are guided by common goals with the EU in ensuring the quality of medicines, and regulatory infrastructure in the efficiency and effectiveness of quality assurance methods. Joint efforts, adherence to international standards and continuous improvement are necessary to improve the quality of medicines in both regions.

Comparison of Quality Assurance Methods for Medicines in Ukraine, the EU and the USA

When comparing the methods of quality assurance in Ukraine with those in Europe, in particular the EU, several differences and similarities are revealed. In Europe, drug quality control testing is governed by comprehensive rules and guidelines set by the European Medicines Agency (EMA) and the European Directorate for Quality in Medicines and Healthcare (EDQM). These rules set out specific requirements for test methods, specifications and quality control acceptance criteria. On the contrary, Ukraine has its regulatory framework controlled by the State Administration on Medicines and Drugs Control, which may have different testing requirements and standards compared to Europe [29, 30].

The analysis showed that both Ukraine and Europe adhere to GMP standards to ensure the quality of pharmaceutical production. However, the implementation and enforcement of GMPs in Europe may be more stringent due to the developed pharmaceutical industry and regulatory infrastructure. GMP compliance in Ukraine and Europe differs in several key aspects. The differences in quality assurance between Ukraine and the EU start with the regulatory framework. For instance, in Europe, GMP compliance is regulated by EU directives and regulations, including the EU GMP Guidelines. On the contrary, Ukraine has a domestic set of GMP rules, which is controlled by the State Administration on Medicines. Although Ukraine strives to bring its rules in line with international standards, there are some differences in the interpretation and implementation of the rules compared to EU standards [31, 32]. The importance of GMP compliance is also confirmed by N.T. Do *et al.* [33].

When comparing the frequency of inspections, in Europe, the competent authorities conduct regular and thorough inspections of manufacturing facilities for compliance with GMP rules. These inspections are usually based on a risk assessment and may include both scheduled and unannounced visits [34]. According to the results of the study by S. Zhang & L. Zhu [35], in the context of different structures of distribution channels, quality control of medicines in the pharmaceutical sector is a common problem faced by all countries of the world, even very developed ones. There is a need to improve the reward and punishment mechanism to improve the pharmaceutical supply and to encourage state regulatory authorities to strictly control pharmaceutical enterprises and provide high-quality medicines to the population.

The comparison shows some differences in the mechanisms for ensuring and complying with GMP rules. In Europe, failure to comply with GMP regulations can cause serious consequences, including regulatory measures such as suspension or revocation of manufacturing licences, product recalls or legal sanctions. The EU has established robust enforcement mechanisms. In Ukraine, despite the existence of regulatory consequences for non-compliance, enforcement may be less consistent or strict compared to Europe, potentially leading to different levels of compliance by producers [36]. European manufacturers have access to a wealth of training resources, guidance documents and industry best practices to support GMP compliance. In addition, European regulators provide support and guidance to manufacturers to help them meet GMP requirements. Despite limited resources and infrastructure issues that may affect the availability and quality of support for manufacturers, Ukraine has a high level of GMP compliance. It is worth noting that due to the efforts to provide training and consultancy, employees of Ukrainian pharmaceutical companies are constantly learning, not only in Ukraine but also abroad. Employees in the pharmaceutical industry are

trained to comply with GMP rules in their work and strictly adhere to them [37].

The above data is confirmed by the results of the study by L. Deshko *et al*. [38], which shows that Ukraine and Europe are striving to ensure GMP compliance to maintain the quality and safety of pharmaceutical products, differences in the regulatory framework, inspection methods, compliance mechanisms and access to resources may lead to differences in the level of compliance between the two regions. Europe usually has a wider network of quality control laboratories than Ukraine, which allows for more thorough and frequent testing of medicines. This can provide a higher level of confidence in the quality and safety of pharmaceutical products [39]. European countries generally have access to advanced testing methods and modern equipment for drug quality testing. This provides a wide range of analytical methods, including chromatography, spectroscopy and mass spectrometry, to be used to accurately assess drug quality [40]. In Ukraine, despite efforts to introduce modern testing methods, limited resources and infrastructure issues can affect the availability and sophistication of test equipment.

In the EU, quality control laboratories for medicinal products are often accredited or certified following international standards such as ISO/IEC 17025 [41]. Accreditation ensures that laboratories operate competently and produce reliable results. In Ukraine, despite efforts to improve the quality of testing laboratories, accreditation may not be as widespread or standardised as in Europe. European regulatory authorities established sampling and analysis protocols for pharmaceutical products to ensure that representative samples are tested, and accurate results are obtained. In Ukraine, although similar protocols may exist, differences in implementation and compliance with these protocols may arise due to limited resources and other factors [42, 43]. European countries actively cooperate with the EU to harmonise the methods and standards of drug quality control testing. This cooperation promotes consistency and compatibility of testing methods across Member States. In Ukraine, despite efforts to align with international standards and best practices, cooperation and harmonisation may be limited compared to Europe.

In Europe, there is a robust pharmacovigilance system in place to monitor and evaluate the safety of medicines after sale. The difference between pharmacovigilance in Ukraine and Europe lies primarily in the level of development, infrastructure and regulatory framework. Moreover, despite the constant improvement and development of new pharmacovigilance methods in Europe, according to F.S. Tonin *et al*. [44], the presence of impurities in finished products remains a serious problem for the pharmaceutical industry, as they can affect the efficacy and safety of medicines and lead to product withdrawal from the market. Several guidelines and pharmacopoeias guide the complex regulatory requirements; however, they are not always clear or cover specific topics in sufficient depth [45]. Medicinal products in the EU undergo a thorough assessment before obtaining a marketing authorisation, including evaluation of quality, safety and efficacy data. The mutual recognition procedure facilitates the recognition of registration certificates issued by one EU Member State in other Member States, ensuring consistent quality standards across the EU. Cooperation between EU Member States, the EMA and other regulatory authorities to exchange information, harmonise rules and promote best practices in quality assurance. Using these methods, Europe maintains a high level of quality of medicines, guaranteeing the safety and efficacy of pharmaceutical products available to its citizens [46].

Quality assurance of medicines in the United States includes various methods controlled by the Food and Drug Administration (FDA) and other regulatory authorities [47]. Pharmaceutical companies must comply with the GMP regulations set by the FDA. These regulations ensure that medicines are consistently manufactured and controlled to meet quality standards suitable for their intended use. The FDA requires pharmaceutical companies to conduct rigorous testing of raw materials, intermediates and finished drugs to ensure that they meet specifications for identity, potency, purity and quality. This includes testing for impurities, efficacy, dissolution and stability. The FDA reviews and approves New Drug Applications and Abbreviated New Drug Applications before drugs can be marketed in the United States. This includes the evaluation of safety, efficacy and quality data provided by manufacturers. The FDA conducts inspections of manufacturing facilities in the US and other countries to ensure compliance with GMP and other quality standards. Inspections can be scheduled or triggered by specific problems or complaints. The FDA monitors the safety of medicines after sale through various surveillance systems, including the FDA Adverse Event Reporting System (FAERS) [48, 49]. Pharmaceutical companies are obliged to report adverse events, and the FDA investigates safety issues and takes regulatory action if necessary. For medical devices, the FDA applies the Quality System Regulations (QSR), which require manufacturers to establish and maintain quality management systems to ensure that their devices consistently meet quality requirements. The Drug Supply Chain Security Act (DSCSA) requires pharmaceutical manufacturers, distributors and pharmacists to implement systems to track prescription drugs as they move through the supply chain, increasing transparency and reducing the risk of counterfeit or falsified medicines. The FDA educates healthcare professionals and the public about the importance of drug safety and quality through various initiatives, including consumer advisories, educational materials, and outreach programmes. By using these methods, the FDA ensures that medicines available in the US meet strict quality standards, protecting public health and improving patient safety [50, 51].

Table 1 shows the main differences in the quality assurance of medicines in Ukraine, Europe and the USA. The comparison was based on the following parameters: regulatory supervision, production standards, pharmacovigilance, inspections, certification of medicines, quality, and safety (Table 1).

Table 1. Comparison of quality assurance of medicines in Ukraine, the USA and Europe

No.	Indicators	Region		
		Ukraine	Europe	USA
1	Regulatory oversight	State Service of Ukraine on Medicines and Drugs Control	EMA	FDA
2	Production standards	GMP	GMP	GMP
3	Pharmacovigilance	Monitoring and reporting of adverse drug reactions has been in place since 1996. Post-registration surveillance department within the State Enterprise "State Expert Centre of the Ministry of Health of Ukraine"	Monitoring and reporting of adverse drug reactions after sales	FAERS
4	Inspections	Inspections of production facilities by the State Administration on Medicinal Products	Inspections of production facilities and distribution channels by national competent authorities to verify compliance	FDA inspections of manufacturing facilities
5	Certification	Issuing certificates to manufacturers that meet quality standards	GMP certification	Certification FDA
6	Quality	Issuance of certificates to manufacturers that meet quality standards by the State Administration on Medicines	Quality control testing: constant testing of medicines in authorised laboratories	QSR
7	Security	Inspections of production facilities and supply chains to verify compliance with production standards	EDQM	DSCSA

Source: [2; 17; 47]

Overall, although both Ukraine and Europe are committed to ensuring the quality of pharmaceutical products through drug quality control testing, differences in regulatory frameworks, resources, infrastructure and cooperation may lead to differences in the level of complexity, accuracy and consistency of testing methods between countries.

Conclusions

Given the information analysed, it is possible to conclude that Ukrainian quality standards for medicines are in line with European standards and are constantly being improved. Ukraine's pharmaceutical industry is making efforts to align its regulations and standards with those of the EU as part of its integration goals. Legislation in Ukraine is constantly being developed to improve the regulatory framework for pharmaceuticals, including quality standards for medicines. These changes include updating existing rules and adopting new ones to align them with EU standards.

There is a tendency for the Ukrainian pharmaceutical industry to prefer to adopt European guidelines, such as those issued by the EMA and EDQM, to inform on internal standards for medicines, which helps to ensure compatibility with European practices. Together with international organisations, Ukraine's pharmaceutical industry has strengthened its regulatory infrastructure and improved the quality of medicines. Achieving full compliance requires ongoing effort and investment. Leading experts in the Ukrainian pharmaceutical industry are seeking to conclude mutual recognition agreements with the EU, under which both parties agree to recognise each other's systems and standards for regulating medicines. This will facilitate trade and regulatory cooperation while promoting the convergence of standards.

European countries actively cooperate within the EU pharmacovigilance system, exchanging information and best practices to improve pharmacovigilance activities. In addition, Europe, given a robust regulatory framework, participates in international cooperation to ensure the quality of medicines around the world. Ukraine still has limited participation in such cooperation, which may affect its ability to access and share important pharmacovigilance information.

Overall, although Ukraine is working to bring its quality standards for medicines in line with the European ones, achieving full compliance may be a gradual process that requires comprehensive regulatory reforms, technical assistance and cooperation with European partners. Using the experience of the FDA and EMA, the Ukrainian pharmaceutical industry will be able to maintain a high level of quality of medicines, ensuring the safety and efficacy of pharmaceutical products for its citizens. Further research should monitor the development of the quality system for medicines in Ukraine and based on the results of the research, make the necessary changes to the country's pharmaceutical industry to improve the quality control of medicines.

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Conflict of Interest

The author declares no conflict of interest.

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Порівняння методів забезпечення якості лікарських засобів в Україні та за кордоном: огляд літератури

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Анотація. Вивчення методів забезпечення якості лікарських засобів в Україні та у інших країнах важливе для вдосконалення та розвитку фармацевтичної галузі. Метою роботи було порівняти системи якості забезпечення лікарських засобів в Україні та за кордоном. У роботі були використані методи структурно-логічного аналізу, бібліосемантичний та аналітико-синтетичний методи. Співставлення було проведено за допомогою аналізу документів, що видаються у іноземних країнах регулюючими органами, такими як Управління з контролю за продуктами та ліками у Сполучених Штатах Америки або Європейським агентство з лікарських засобів у Європі та відіграють важливу роль у забезпеченні якості ліків. В Україні за нормативний нагляд відповідає Державна служба України з лікарських засобів та контролю за наркотиками. Згідно результатів проведеного аналізу у системах контролю за якістю лікарських засобів в Україні та за кордоном виявлено ряд відмінностей. Стандарти Good Manufacturing Practice загальновизнані для забезпечення якості у фармацевтичному виробництві. Дотримання стандартів є обов'язковим в Україні та за кордоном. Регулярні випробування ліків в уповноважених лабораторіях мають вирішальне значення для забезпечення їхньої безпеки та ефективності. Україна, як і інші країни, проводить тестування контролю якості. Моніторинг та звітність про побічні реакції на ліки після продажу мають важливе значення для виявлення та усунення проблем безпеки. Хоча системи фармаконагляду існують в Україні та за кордоном, можуть існувати відмінності з погляду вимог до звітності, інфраструктури та ресурсів, що виділяються на діяльність з фармаконагляду. Планові перевірки виробничих підприємств та каналів збуту проводяться для перевірки дотримання правил. Просвітництво медичних працівників та громадськості про важливість забезпечення якості лікарських засобів сприяє ухваленню обґрунтованих рішень. З проведеного аналізу контролю якості лікарських засобів можна зробити висновок, що фундаментальні методи забезпечення якості лікарських засобів у всьому світі схожі, відмінності у нормативній базі, ресурсах, інфраструктурі та реалізації можуть вплинути на ефективність та дієвість заходів забезпечення якості між Україною та іншими країнами. Співпраця, дотримання міжнародних стандартів та постійне вдосконалення необхідні для просування практики забезпечення якості в Україні та за кордоном

Ключові слова: органи контролю у медицині; медичні препарати; належна виробнича практика; фармаконагляд; побічні дії

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