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Comparative efficacy of protected beta-lactam therapies for septic shock treatment

Eficacia comparativa de las terapias con betalactámicos protegidos para el tratamiento del shock séptico

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SUMMARY

Gram-negative bacteria pose a significant threat as primary agents of infection in septic shock, a condition with high mortality rates in intensive care patients. To combat this, chemists develop antibiotic drugs fortified with protective agents, such as carbapenemase inhibitors, to counter bacterial resistance mechanisms. This study aims to evaluate the clinical efficacy of two antibiotic combinations, imipenem/cilastatin/relebactam (IMI/REL) and piperacillin/tazobactam (PIP/TAZ), in the treatment of septic shock caused by gram-negative bacteria. Seventeen patients diagnosed with septic shock were divided into Group 1 with IMI/REL and Group 2 with PIP/TAZ. All patients received antishock treatment, renal replacement therapy, and ventilation. Bacteriological detection identified four gram-negative pathogens in biological samples, with further phenotyping and genotyping confirming resistance mechanisms. Both IMI/REL and PIP/TAZ exhibited notable resistance against Acinetobacter baumannii and Pseudomonas aeruginosa. Metallo-β-lactamases and class D carbapenem hydrolysing oxacillinase were detected in Acinetobacter baumannii. Therapy of IMI/REL and PIP/TAZ proved to be ineffectual in patients afflicted with these strains, leading to fatalities. The study highlights the ineffectiveness of IMI/REL and PIP/TAZ in treating septic shock caused by highly resistant gram-negative bacteria. The results of this study are crucial for guiding future clinical trials and strategic decision-making regarding the use of beta-lactam antibiotics combined with inhibitors.

Keywords: *Antishock treatment, Acinetobacter baumannii, antibiotic therapy, genotyping, clinical effectiveness.*

RESUMEN

Las bacterias gramnegativas representan una importante amenaza como agentes primarios de infección en el shock séptico, una afección con elevadas tasas de mortalidad en pacientes de cuidados intensivos. Para combatirlo, los químicos desarrollan fármacos antibióticos enriquecidos con agentes protectores, como los inhibidores de la carbapenemasa, para contrarrestar los mecanismos de resistencia bacteriana. El objetivo de este estudio es evaluar la eficacia clínica de dos combinaciones de antibióticos, imipenem/cilastatina/relebactam (IMI/REL) y piperacilina/tazobactam (PIP/TAZ), en el tratamiento del shock séptico causado por bacterias gramnegativas. Diecisiete pacientes diagnosticados

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con shock séptico se dividieron en Grupo 1 con IMI y Grupo 2 con PIP/TAZ. Todos los pacientes recibieron tratamiento antishock, terapia renal sustitutiva y ventilación. La detección bacteriológica identificó cuatro patógenos gramnegativos en las muestras biológicas, y la fenotipificación y genotipificaciones posteriores confirmaron los mecanismos de resistencia. Tanto IMI/REL como PIP/TAZ mostraron una notable resistencia frente a Acinetobacter baumannii y Pseudomonas aeruginosa. En Acinetobacter baumannii se detectaron metalo-β-lactamasas y oxacilinasas hidrolizadoras de carbapenemasas de clase D. El tratamiento con IMI/REL y PIP/TAZ se basó en el análisis genotípico. El tratamiento con IMI/REL y PIP/TAZ resultó ineficaz en los pacientes afectados por estas cepas, lo que provocó víctimas mortales. El estudio pone de relieve la ineficacia de la IMI/REL y la PIP/TAZ en el tratamiento del shock séptico causado por bacterias gramnegativas muy resistentes. Los resultados de este estudio son cruciales para orientar futuros ensayos clínicos y la toma de decisiones estratégicas en relación con el uso de antibióticos betalactámicos combinados con inhibidores.

Palabras clave: *Tratamiento antishock, Acinetobacter baumannii, antibioticoterapia, genotipado, eficacia clínica.*

INTRODUCTION

Septic shock continues to be a major global cause of death in Intensive Care Units (ICU), impacting both developed and developing countries. Sepsis is a severe systemic reaction to infection that can result in septic shock and multiple organ failure. The elderly, children, pregnant women, new mothers, and immunocompromised people are most prone to this condition. Most nosocomial bacteria that cause septic shock in intensive care unit patients also show a high level of antibiotic resistance. The purpose of this study is to examine the efficacy of piperacillin/tazobactam (PIP/TAZ) and imipenem/cilastatin/relebactam (IMI/REL) in treating ICU patients with septic shock.

As Piccioni et al. (1) note, the development of septic shock in intensive care patients is still a leading cause of death in many cases, both in developed and developing countries. The risk of sepsis appearing as a complication of the main disease flow is especially high amongst elderly patients, children, pregnant, parturient, and patients with low immunity status.

Septic shock is caused by one of the types of sepsis. Sepsis is defined as a life-threatening organ dysfunction caused by the improper, inadequate reaction of a body to the infection pathogen (2). According to the International Guidelines for Management of Sepsis and Septic Shock from 2021 by Evans et al. (3), septic shock is usually characterized by major dysfunctions of blood circulation and cellular metabolism, significantly increasing the odds of the lethal outcome.

A quick Sequential Organ Failure Assessment scale (qSOFA) is recommended to diagnose sepsis and septic shock and evaluate organ dysfunction dynamics. If the qSOFA scale is >2, then it is recommended to begin the sepsis treatment procedure. The following clinical characteristics are regarded as criteria for qSOFA:

- The Glasgow Coma Scale (evaluation for consciousness dysfunction) is less than 15;
- Systolic blood pressure (SBP) is less than 100 mmHg;
- The breathing rate is higher than 22 inhales per minute.

2-3 points on the qSOFA scale are noted to end up in a lethal outcome in 70 % of cases but only in 24 % of patients with confirmed infection. As such, Perner et al. (4) offer to use other criteria for sepsis and septic shock diagnostics in intensive care:

- body temperature is 38°C and higher or 36°C or lower.
- leucocytosis or leukopenia.
- neutropenia.
- high lactate rates in blood plasma.
- high procalcitonin rates.

Septic complications and bacillaemia are most often caused as a result of intensive care patient infection by nosocomial pathogens. The primary peculiarity of these pathogens is their complete or partial resistance to antibiotics. According to Di Franco et al. (5), four types of

gram-negative bacteria are primarily responsible for the development of septic shock, these are *Klebsiella pneumoniae (K. pneumoniae)*, *Escherichia coli (E. coli)*, *Acinetobacter species (A. species)*, and the *Pseudomonas aeruginosae (P. aeruginosae)*. The carbapenemase resistance mechanism formation of these microorganisms has been thoroughly researched. Genes that code the carbapenemase are on the mobile genetic elements alongside other genes, which form resistance to different antibiotics (6). Idowu et al. (7) note that it is due to this that the gram-negative bacteria develop multi-resistance to all β-lactam antibiotics.

The latest antimicrobial surveillance report for the European Center for Disease Prevention and Control (ECDC) (8) found a trend of rising carbapenem resistance across Europe. This tendency, according to Nowaczyk et al. (9), was observed with *K. pneumoniae*, for which the proportion of invasive isolates was 8.2 % in 2020 compared to 2.1 % in 2016, and *Acinetobacter species* – 78.2 % in 2020 compared to 66 % in 2016. Dhaese et al. (10) recommend bacteriological examination of ICU patient samples for the differential diagnosis of sepsis and individual selection of antibiotic therapy. However, implementing traditional cultural methods takes 1-2 days (11). Determining antigens using the enzyme-linked immunosorbent assay (ELISA) method and the pathogen's genetic material using polymerase chain reaction (PCR) takes several hours (12).

An analytical study of Strich et al. (13) proves that every hour of delaying septic shock antibiotic therapy increases the chances for the lethal outcome proportionately. In these cases, ICU doctors are forced to prescribe antimicrobic medications empirically based on their experience and intuition (14). Modern pharmaceuticals offer combined protected antibiotic medications, which include beta-lactam and bacterial beta-lactamase inhibitors. In their analysis, Veiga and Paiva (15) highlight relebactam (REL) and tazobactam as microorganism ferment inhibitors currently widely used.

This study's main objective is to assess the efficacy of two inhibitor-protected beta-lactams in treating septic shock in ICU patients: IMI/REL and PIP/TAZ. Research tasks were developed:

- to provide insight into the effectiveness of the antibiotics against nosocomial infections, which are often caused by multi-resistant gram-negative bacteria like *Klebsiella pneumoniae*, *Escherichia coli*, *Acinetobacter species*, and *Pseudomonas aeruginosa*.
- to Analyse the resistance patterns of the bacteria producing septic shock is another crucial goal of the research.
- to assess parameters including death rates, incidence of acute respiratory distress syndrome (ARDS), acute renal failure (ARF), and the overall efficacy of anti-shock therapy.

MATERIALS AND METHODS

This is a retrospective, uncontrolled, non-randomized, observational study in the ICU at the University Hospital in Krakow, Poland. The institution's ethics committee approved the protocol. Each patient or their relative signed an informed consent form to participate.

At the preparatory stage, the study included 17 patients with septic shock who were treated during 2021-2022 in the ICU. The exclusion criteria were age less than 18 years, pregnancy or lactation, previous use of any of the prescribed drugs, allergy to any of the components of these drugs, meningitis or cystic fibrosis, inability to sign informed consent or absence of relatives, dementia, participation in clinical trials in the previous six months.

The stage of material collection and observation was concluded 96 hours from the estimated moment of septic shock development after the patient's admission to the ICU. Demographic characteristics were collected: age, gender, weight, body mass index, comorbidities, main disease, and treatment methods for the main disease.

Patients were randomly divided into two groups: group 1 received IMI/REL (n=9), and group 2 received PIP/TAZ (n=8). Moreover, all patients underwent appropriate anti-shock therapy, which included noradrenaline preparations and infusions of crystalloid and albumin solutions. ARDS was diagnosed among five patients, because of which artificial lung

ventilation was employed. Among seven patients, symptoms of ARF were found, in connection with which they were prescribed renal replacement therapy (RRT), which is a procedure of venous hemofiltration. Also, at this stage, data on the number of lethal and positive outcomes of the septic shock treatment and the causes of mortality was collected.

Initial IMI/REL infusion was administered each 6 hours with 500 mg of imipenem/cilastatin (IMI) and 250 mg of the REL for patients with normal creatinine clearance. PIP/TAZ infusion was administered at 4.5 g piperacillin and 0.5 g of tazobactam every 6 hours with lengthy infusions for 3-4 hours. Henderson et al. (16) have published the clinical test results, again proving the need for the medication dose estimation to overcome minimum inhibiting concentration (MIC) in blood four times.

Biomaterial was taken from all patients for a general blood test and the study of the lactate level, procalcitonin, and creatinine clearance. Determination of MIC, sensitivity, and antibiotic resistance of infectious pathogens was carried out by unified cultural and disk diffusion methods and using E-tests in fresh cultures of isolates (17). For phenotyping, the CarbaNP test was used with various modifications. Genotyping of multidrug-resistant isolates was performed using real-time multiplex PCR.

At the statistical processing stage of the material, the STATISTICA 10.0 program by StatSoft was used. For each quantitative indicator, the median and the interquartile range were determined.

RESULTS

Most of those examined were males over 60 who were transferred to the ICU after surgery (Table 1). The causes of surgery were cardiovascular pathologies, injuries of soft tissues and organs of the chest, and cirrhosis of the liver. The most common comorbidities were type II diabetes mellitus (in 3 patients, 18 %) and alcoholism (in 2 patients, 12 %). Only one patient from the research basis (6 %) has a body mass index (BMI) corresponding to the normal body weight. Among eight patients (47 %), BMI varied from 25 to 29, indicating excessive weight, while among the remaining eight patients (47 %), BMI ranged from 30 to 33, typical for grade I obesity.

Table 1

Clinical and demographic characteristics of patients

Characteristic	n, (%) or median (interquartile diapason)	
	Group 1, (IMI/REL), n=9	Group 2, (PIP/TAZ) n=8
Males	6 (67)	6 (75)
Age, years	66 (54-70)	71 (62-79)
Weight, kg	91 (81-95)	88 (74-104)
Body Mass Index	29 (26-31)	30 (26-31)
qSOFA	3 (2-3)	3 (2-3)
Lactate, mmoL/L	3.1 (2.8-4.1)	3.6 (3.3-4.0)
Procalcitonin, ng/mL	21.4 (9.7-55.1)	31.9 (11.6-57.8)
Bacillaemia	4 (44.44)	4 (50)
ARDS	3 (33.33)	2 (25)
Acute Kidney Failure (AKF)	3 (33.33)	4 (50)
Lethal outcome	5 (55)	4 (50)

During observation in the ICU, all examined patients showed an increase in plasma lactate level by 1.5-2 times compared with the norm (0.5-2.2 μmol/L). The median content of procalcitonin in group 1 was 21.4 ng/mL, 40 times higher than the normal value (<0.5 ng/mL). In Group 2, the

median value was 60 times higher than normal, equalling 31.9 ng/mL.

The septic shock diagnosis was established using the qSOFA scale: among 16 patients; there was a sharp decrease in SBP to 65-70 mmHg, 15 patients had an increase in respiratory rate, 12 patients had a Glasgow Coma Scale score of fewer than 15 points. The most common complications were ARDS and ARF.

All patients with acute respiratory distress syndrome underwent mechanical ventilation. Mild ARDS was diagnosed among three patients, and non-invasive mechanical ventilation was employed as respiratory therapy. Among two patients, ARDS of moderate severity was noted, and the value of the PaO_2/FiO_2 oxygenation index was approximately 150 mmHg. Invasive mechanical ventilation with lung protection, including the prone position for at least 16 hours, was performed for those patients. The target tidal volume was 6-8 mL/kg of body weight. In approximately half of the patients, bacillaemia was found.

The formation of acute renal failure was evaluated by the level of creatinine in the blood serum and the glomerular filtration rate (creatinine clearance) in the first few hours after the expected development of septic shock (Table 2). AKF was diagnosed among three patients in Group 1. Among them, the median creatinine content exceeded the normal values by more than three times, while the creatinine clearance decreased almost twice. In group 2, acute renal failure was diagnosed among four patients. The creatinine concentration in these patients was also three times higher than normal, and the clearance fell by two times; among one patient in Group 1 and 2 patients in Group 2, acute renal failure developed against the background of ARDS. In Group 1, the patient had ARDS of moderate severity; in Group 2, 1 patient had moderate severity, and one patient had mild severity. All patients with acute renal failure were prescribed RRT, a procedure of venous hemofiltration. Since 6 out of 7 examined patients had elevated body mass index values, they underwent prolonged high-volume hemofiltration for 48 hours, with a 50 mL/kg/h filtration dose. Alongside RRT, for patients in Group 1, the antibiotic IMI/REL concentration was reduced to 400 mg of IMI and 200 mg of REL due to confirmed nephrotoxicity.

Table 2

Evaluation of kidney functionality amongst patients with septic shock development (median, (interquartile diapason))

Group 1, (IMI/REL), n=9				Group 2, (PIP/TAZ) n=8			
AKF is absent, n=6 (66.66 %)		AKF is present, n=3A (33.33 %)		KF is absent, n=4 (50 %)		AKF is present, n=4 (50 %)	
Creatinine, mmol/L	Creatinine clearance,	Creatinine, mmol/L mL/min	Creatinine clearance,	Creatinine, mmol/L mL/min	Creatinine clearance,	Creatinine, mmol/L mL/min	Creatinine clearance, mL/min
78 (75-81)	116 (106-120)	266 (241-275)	60 (55-62)	77 (71.5-81)	111 (94-123.5)	270 (257-285)	51.5 (49-57)

Biomaterial was collected for bacteriological studies before initiating antibiotic therapy in patients of both groups. Since delaying antibiotic therapy substantially increases the likelihood of death, IMI/REL or PIP/TAZ infusions were started as soon as the diagnosis of septic shock was made.

Nosocomial infections were found among all examined patients. More than one infectious agent was present among four patients in Group 1 and three patients in Group 2 (Table 3). Gram-negative bacteria predominated in the spectrum of pathogens. One type of gram-positive bacteria was present in patients in Group 1 and

Group 2. The identification of *Pseudomonas aeruginosae* and *Acinetobacter baumannii* in purulent tracheobronchial secretions in patients undergoing mechanical ventilation indicated the development of ventilator-associated pneumonia. After abdominal surgery, *Escherichia coli* was isolated in 1 patient, and *Klebsiella pneumoniae* was isolated in 1 patient, which confirms the development of intra-abdominal infection. In patients with *Bacillaemia*, *Klebsiella pneumonia* was observed in 4 cases, *Escherichia coli* in two instances, and *Acinetobacter* in 2 cases, further aggravating the severity of the condition.

Table 3

The spectrum of invasive isolates among patients with septic shock

Pathogen	Group 1, (IMI/REL), n=9	Group 2, (IMI/REL) n=8
Pseudomonas aeruginosae, n (%)	2 (22.2)	2 (25)
Klebsiella pneumoniae + Acinetobacter baumannii, n (%)	1 (11.15)	1 (12.5)
Pseudomonas aeruginosae + Acinetobacter baumannii, n (%)	3 (33.3)	2 (25)
Escherichia coli, n (%)	2 (22.2)	2 (25)
Streptococcus pneumonia, n (%)	-	1 (12.5)
Staphylococcus aureus, n (%)	1 (11.15)	-

In the work by Matuschek et al. (18) testing of detected infectious agents was under the European Committee on Antimicrobial Susceptibility Testing guidelines. Serial dilution MIC values were used to determine breakpoint cut-off points for wild-type organisms (organisms without phenotypically detectable resistance) and to calculate sensitivity. The MIC for IMI/REL ranged from 0.125 mg/L for *Staphylococcus aureus* to 2 mg/L for *Pseudomonas aeruginosae*. In the case of PIP/TAZ, *breakpoint cut-off* points for the *Acinetobacter baumannii* were not defined, and the disk diffusion method according to Clinical & Laboratory Standards Institute (19) standards with a PIP/TAZ content per disk of 100 μg was used to study resistance. For other pathogens, the PIP/TAZ MIC ranged from 0.064 mg/L for *Streptococcus pneumoniae* to 16 mg/L for *Pseudomonas aeruginosae*.

A. baumannii strain with high resistance to the first-generation antibiotics, the protected imipenem, and to the comparative medication, as well as to the inhibitor-protected piperacillin and the amoxicillin/clavulanate and the ceftriaxone (Table 4). Notably, high resistance to β-lactams was prominent in the invasive *P. aeruginosae* isolates. The acquired data allowed us to conclude that the presence of multidrug resistance (MDR) in *Acinetobacterium* and *Pseudomonas aeruginosa*, the pathogens of nosocomial infections, was present amongst the examined patients with septic shock. On the contrary, high sensitivity to the studied combined antibiotic medications was prominent for *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Streptococcus pneumonia* (20). Those were present in Group 1.

Based on acquired data, the carbapenemase production for the multiresistant strains *P. aeruginosae* and *A. baumannii* was suggested. Phenotypic research of invasive isolates was carried out to determine what type of β-lactamase they produce. Since the studied strains, during the evaluation of antibiotic resistance, have shown insensitivity to amoxicillin/clavulanate, phenotyping of extended-spectrum β-lactamase (ESBL) was not performed. Additional bacterial enzyme detection was employed using a modified Carba NP II test with specific inhibition. As such, the use of tazobactam in the test made it possible to assess the activity of class A β-lactamases, KRS-type, and the use of EDTA allowed the assession of the activity of metallo-β-lactamases (MBL).

Table 4

Antibiotic resistance of invasive isolates among patients with septic shock

Pathogen		*P. aeruginosae*	*A. baumannii*	*E. coli*	*K. pneumoniae*	*S. aureus*	*S. pneumoniae*
Group 1, (IMI/REL), n=9	Sensitivity (S), %	51	6.5	96	90	97	98
	Intermediate resistance (I), %	19	10.5	4	2.5	3	2
	Resistance (R), %	30	83	0	7.5	0	0
Group 2, (PIP/TAZ) n=8	Sensitivity (S), %	45.5	10	97	89	97	97.5
	Intermediate resistance (I), %	22	9.5	3	2	3	2.5
	Resistance (R), %	32.5	80.5	0	9	0	0

As a result, the production of two enzyme types, bovine and MBL, was confirmed in the analysed *P. aeruginosae* strains (21).

During the analysis of *A. baumannii*, the CarbAcineto NP test, specially designed for the phenotyping of this pathogen, was used. As such, the production of OXA-23-like carbapenemase was noted in the analysed isolates. All detected strains were analysed to identify the genes that cause ferment synthesis. Amongst 3 (43 %) studied strains of *Acinetobacterium*, two families of genes encoding different series of enzymes were found. Genotyping indicated the presence of the *OXA-23-like* gene, which causes the synthesis of carbapenem-hydrolysing class D oxacillinase and *Verona integron-encoded metallo-beta-lactamases* (VIM-1), encoding metallo-β-lactamases. In 2 cases, only *OXA-23-like* genes were identified, in one case, the *VIM-1* gene, and in another, the *IMP-1* gene responsible for imipenemase synthesis.

During the genotyping of the *P. aeruginosa*, 3 (30 %) strains had *IMP-1* gene, while in 2 other cases, a *VIM-1* gene was found. The fatal outcomes in both groups after 96 hours of observation were approximately the same, about 50 % of cases. Among patients with septic shock complicated by ventilator-associated pneumonia, the mortality rate was 40 % (2 patients). Among patients with acute renal failure, despite ongoing RRT, the mortality rate was 29 % (2 patients). Among five examined patients, the development of multiple organ failure syndromes (MOS) was noted, which resulted in a fatal outcome (22). All of the examined patients had cardiovascular insufficiency. In 1 patient, it was accompanied by liver failure. In another patient, bowel dysfunction progressed. Among 3 cases, there was severe cerebral dysfunction and the occurrence of secondary cerebral disorders. Among 7 cases of lethal outcomes in patients, strains of multiresistant *Acinetobacter baumannii* were detected, and in two instances – *Pseudomonas aeruginosae*.

An attempt to evaluate the benefits of prescribing these antibiotics to ICU patients was concluded. Patients were randomly divided into two groups, while randomization and blind placebo control were not carried out. The obtained clinical and demographic characteristics of the patients indicated that the formation of two homogeneous groups in terms of age, sex, laboratory parameters, and severity of the condition was achieved. It should also be noted that all patients received adequate anti-shock therapy, which included crystalloid, albumin solution, preparations of norepinephrine, dobutamine, and hydrocortisone infusions following individual prescribed needs.

A study examining patients with advanced age, multiple health issues, and varying degrees of obesity found that only one patient had a normal BMI. All patients exhibited elevated plasma lactate levels and higher procalcitonin levels, indicating severe infections and septic shock. Common complications included ARDS

and ARF. Mechanical ventilation was necessary for all patients with ARDS, and bacillaemia was detected in about half of the patients. RRT was administered to all patients with ARF, highlighting the critical nature of renal support. Bacterial studies revealed the presence of nosocomial infections in all patients, with gram-negative bacteria, particularly *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, associated with ventilator-associated pneumonia and intra-abdominal infections. Antibiotic resistance profiling showed that *Acinetobacter baumannii* and *Pseudomonas aeruginosa* exhibited high resistance to several antibiotics, while *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus pneumoniae* showed high sensitivity to the combined antibiotic medications studied. Despite aggressive treatments, the mortality rate remained high, with septic shock complicated by ventilator-associated pneumonia or multiple organ failure being particularly vulnerable.

DISCUSSION

Inhibitor-protected beta-lactams are widely used in modern clinical practice. According to the recommendations of the European Society for Clinical Microbiology for the treatment of nosocomial infections, compiled by Paul et al. (23), it is offered to use the combination of IMI/REL or PIP/TAZ medications. According to Heo (24), these medications have shown high efficacy against the main pathogens of nosocomial infections – gram-negative bacteria *in vitro*. However, the success of their clinical use in treating severe infectious diseases such as sepsis and septic shock remains insufficiently studied.

Changes in the level of procalcitonin in the blood are an independent prognostic marker for developing sepsis and septic shock. The conclusions of Hu and Zhang (25) confirm that an increase in the content of the marker above ten ng/mL indicates not only a severe case of septic shock but also a high probability of developing complications, including acute renal failure. Among the patients of the current research, the values of the median concentration of procalcitonin from 21.4 ng/mL to 31.9 ng/mLwere observed, which is 2 and 3 times higher than the prognosis value.

As RRT, a venous hemofiltration procedure was prescribed with increased serum creatinine by more than three times and a simultaneous decrease in glomerular filtration rate. Bacteriological examination revealed *E. coli* in the urine of 4 patients and the blood of 2 patients. The high sensitivity of this pathogen to the analysed drugs and the rapid initiation of treatment had a positive effect on the outcome of patients with septic shock complicated by acute renal failure. Mortality was 29 %, which correlates with data from other studies, such as Fish et al. (26). Fatal outcomes were observed in patients with confirmed *Pseudomonas aeruginosa* and *Acinetobacter* with a high level of polyresistance.

Another common complication during septic shock is acute respiratory distress syndrome. The presence of ARDS is one of the risk factors for the development of ventilator-associated pneumonia (VAP). In the case of this study, VAP was induced by multiresistant strains of *Acinetobacter baumannii* and *Pseudomonas aeruginosae*. The pronounced resistance of pathogens to the studied antimicrobial drugs led to a lethal outcome among two patients with VAP. According to Guillamet et al. (27), the reason for the development of polyresistance in *Pseudomonas aeruginosa* and acinetobacteria, which results in high mortality in patients with bacterial pneumonia, may be inadequate antibiotic therapy at the initial stages of the disease. However, patients who had previously taken the studied drugs (the IMI/REL and PIP/TAZ) were not included in the research.

As a result of MDR-type infectious pathogens phenotyping, the production of class A and MVL-type β-lactamases in *P. aeruginosae*, and *OXA-23-like* type carbapenemase in *A. baumannii* was confirmed. According to Tamma and Simner (28), currently used methods for β-lactamase phenotyping production in *Acinetobacterium* have several significant drawbacks, including unsatisfactory sensitivity and specificity, in the test used in the latter study. Therefore, the authors of the second study decided to perform the genotyping procedure for isolated invasive isolates.

The procedure resulted in the detection of genes that cause the synthesis of metallo-β-lactamases in *P. aeruginosae*. An even greater diversity emerged due to the genotyping of *A. baumannii*.

Some of its strains have demonstrated the ability to synthesize both metallo-β-lactamases and oxacillinases simultaneously. A pharmaceutical review by Mansour et al. (29) describes IMI/REL as a combination drug containing imipenem, an antibiotic of the β-lactam group, cilastatin, an inhibitor of dehydropeptidase-I in the kidneys, and REL, an inhibitor of class A and C bacterial β-lactamases. A decrease in the activity of imipenem metabolism in the kidneys prolongs the duration of its action. Lob et al. (30) studied the inhibitory effect of relbactam *in vitro* and *in vivo* and showed that it is ineffective against metallo-β-lactamases and oxacillinases. However, the genes of just these enzymes were discovered during the genotyping of *Acinetobacter baumannii* and *Pseudomonas aeruginosae*.

García-Fernández et al. (31) conducted a multicenter sensitivity assessment of *P. aeruginosae* and *A. baumannii* to the combined reference medication PIP/TAZ, which contains a semi-synthetic ureidopenicillin, piperacillin, and several types of β-lactamase inhibitor, tazobactam. It is known that tazobactam exhibits high activity against class A β-lactamases and class D metallo-β-lactamases. However, Lukić-Grlić et al. (32), who have investigated strains of bacteria producing carbapenem-hydrolysing class D oxacillinases, have not confirmed PIP/TAZ activity *in vivo*. During the genotyping of *Acinetobacter baumannii* strains, genes encoding these enzymes were identified in 5 cases.

Coyne et al. (33) attribute the high resistance of *A. baumannii* to protected carbapenems attributed to additional manifestations of resistance, such as the activation of genes encoding efflux pumps. Among them, the expression of genes of the superfamily of RND pump proteins plays the greatest role since they can pump antibiotics through the microorganism ‘s internal and external membranes. In a review by Lambden et al. (34), it is noted that high mortality due to septic shock remains one of the main problems in the management of patients in intensive care units. The rapid development of multiple organ failure syndrome (MOS) in immunocompromised patients is still ahead of the ability of physicians to counteract such consequences. During the analysis of causes of MOS in the patients, attention was drawn to the fact that cerebral disorders (3 cases, 30 %) are in second place in frequency after the development of cardiovascular insufficiency (5 patients, 50 %). According to Quinton et al. (35), high doses of PIP/TAZ in the blood of patients can lead to neurological complications among patients with septic shock due to the proven toxicity of this medication. The results do not allow us to either confirm or refute this conclusion since the examined patients had impaired consciousness according to the Glasgow Coma Scale even before the start of antibiotic therapy.

The main challenge for septic shock antibiotic therapy, caused by the nosocomial infection, remains the multi-resistance of *Acinetobacter baumannii* (36). Mutations in the genome leading to reduced or increased gene expression, as well as the ability for horizontal gene transfer, provide *A. baumannii* the ability to synthesize β-lactamases, metallo-β-lactamases, and extended-spectrum beta-lactamase (ESBL), *plasmid-mediated AmpC beta-lactamases*, carbapenem-hydrolyzing class D oxacillinases. In their review, Bonnin et al. (37) note that such a variety of enzymes leads to the formation of pathogen resistance to aminoglycosides, broad-spectrum cephalosporins, carbapenems, tigecycline, and colistin, which are antibiotics of last resort.

In the current research, an attempt to evaluate the benefits of prescribing these antibiotics to ICU patients was concluded. Patients were randomly divided into two groups, while randomization and blind placebo control were not carried out. The obtained clinical and demographic characteristics of the patients indicated that the formation of two homogeneous groups in terms of age, sex, laboratory parameters, and severity of the condition was achieved. Accordingly, it is quite reasonable to compare the effectiveness of antibiotics in patients in these groups. It should also be noted that all patients received adequate anti-shock therapy, which included crystalloid, albumin solution, preparations of norepinephrine, dobutamine, and hydrocortisone infusions following individual prescribed needs.

Unfortunately, the number of resistant strains of microorganisms producing carbapenemase, which are not inhibited by employment medications, is increasing worldwide. The growing popularity of medical tourism and

the uncontrolled use of antibiotics in some countries explains this phenomenon. To provide more conclusive findings, future studies should concentrate on the therapeutic effectiveness of these antibiotics in bigger, controlled trials. To overcome MDR pathogen resistance, different medicines and combinations must be investigated. Furthermore, improving results may be possible by developing specific therapies and comprehending the genetic underpinnings behind resistance. Along with methods to slow the development of MDR pathogens, more research should be done on the role that early and sufficient antibiotic treatment plays in preventing the formation of MDR pathogens.

CONCLUSIONS

The number of positive clinical and microbiological responses in groups 1 and 2 did not differ significantly and amounted to 45 % (4 outcomes) and 50 % (4 outcomes), respectively. Therefore, the data of this research do not support the benefit of IMI/REL or PIP/TAZ in patients with septic shock in the ICU. Inhibitor-protected beta-lactams showed high efficacy in the treatment of sepsis caused by *Escherichia coli* and *Klebsiella pneumoniae*, moderate efficacy against *Pseudomonas aeruginosae*, and insignificant efficacy against multidrug-resistant *Acinetobacter baumannii*. As some patients had more than one MDR-type infectious agent, IMI/REL and PIP/TAZ did not produce a positive result in such cases.

The pronounced resistance of *P. aeruginosae* to the IMI/REL medications is caused by the presence of a phenotype in it, characterized by the production of metallo-β-lactamases, which are not inhibited by relbactam. Tazobactam, which is part of PIP/TAZ, is effective against these enzymes. A higher degree of a positive outcome in group 2 was expected, yet among some patients of this group, alongside the multidrug-resistant strain of *Pseudomonas aeruginosa*, *A. baumannii* MDR-type was detected. A feature of the genotype of the established strain was the existence of *OXA-23-like* genes for carbapenem-hydrolyzing class D oxacillinases. Tazobactam is not an inhibitor for this type of bacterial enzyme; therefore, in patients with confirmed *A. baumannii*, the administration of PIP/TAZ did not achieve the desired results.

The problem of early detection of infectious pathogens in patients with septic shock is currently of substantial actuality. Modern genotyping methods using real-time multiplex PCR enable a detailed characterization of the causes of multi-resistance in the identified strains. Proper provision of such information would allow the anesthesiologist-resuscitator to prescribe antibiotic therapy not empirically but purposefully, considering the characteristics of the detected microorganisms.

Multi-resistance strains of nosocomial infections are still one of the primary causes of lethal outcomes during sepsis. Employment of protected beta-lactams only partially solves the problem.

The study has several limitations that impact the generalizability and robustness of its findings. Firstly, the research was retrospective, uncontrolled, and non-randomized, which inherently introduces biases and limits the ability to establish causal relationships. Additionally, the sample size was relatively small, with only 17 patients included in the study. Consequently, the conclusions drawn from this study may not be applicable to all patients in different settings or with varying underlying conditions. The lack of detailed information on factors such as the severity of comorbid conditions, variations in the implementation of anti-shock therapies, and differences in ICU care practices makes it difficult to attribute differences in outcomes solely to the antibiotics used.

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