

loma virus co-infection, no clinical depression and sleeping disturbance. Relative telomere length (RTL) was determined by Real-time polymerase chain reaction from mononuclear cells. Genomic DNA was isolated from mononuclear cells, relative to a unique reference sequence by salting out method.

**RESULTS:** The mean age was  $42.7 \pm 13.2$  years. The mean RTL was  $2.50 \pm 1.87$ . The total duration of HIV infection averaged  $92.4 \pm 72.2$  months. Patients were receiving cART for an average of  $82.02 \pm 64.81$  months. The Kendall correlation test showed that cART do not affect preterm RTL shortening in PLWHIV ( $p=0.002$ ) with HIVRNA plasma-viral-load below 50 copies/mL.

**CONCLUSIONS:** The timely introduction of successful cART does not affect preterm telomere shortening.

### The fight against gram-negative superbugs in the intensive care units

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**AIM:** To determine the factors affecting mortality in patients with gram-negative bloodstream infections (GNBSI) in intensive care units (ICU).

**BACKGROUND:** Mortality and morbidity from GNBSI are high due to the increasing antimicrobial resistance of gram-negative pathogens and the limited access to available antibiotics. A comprehensive understanding of the associated factors is therefore essential to reduce mortality.

**METHODS:** The study was conducted retrospectively in Akdeniz University Hospital, Turkiye, between January 2020 and December 2022.

**RESULTS:** Data from 202 patients with GNBSI in the ICU were analysed. Of the patients 65.8% were male. Of the patients 70.7% died. Mortality rates were lower in ICU admissions due to neurological disease and trauma ( $p=0.001$ ), in those who had a tracheostomy ( $p=0.001$ ) and were fed with a percutaneous endoscopic gastrostomy ( $p=0.025$ ). Mortality rates were higher in patients in isolated rooms ( $p=0.003$ ). The analysis indicated that older age ( $p=0.01$ ), concomitant pulmonary diseases ( $p<0.001$ ), neutropenia ( $p<0.001$ ), abdominal surgery ( $p=0.025$ ) and previous antibiotic use ( $p=0.022$ ) were associated mortality. A total of 250 organisms were identified in the blood cultures. The most common bacteria were *Klebsiella pneumoniae* ( $n=112$ , 44.8%) and *Acinetobacter species* ( $n=83$ , 33.2%). Of all isolates 85.2% were multidrug resistant. Carbapenem resistance was higher in non-survivors ( $p<0.001$ ). The C-reactive protein/albumin ratio calculated on admission was higher in those who died ( $p=0.004$ ).

**CONCLUSIONS:** In this specific patient group, it is of the paramount importance to identify predisposing factors, administer an appropriate antibiotic and provide supportive therapies in order to reduce mortality.

### Plasma-activated water efficacy in the management of experimental wounds infected with *Staphylococcus aureus* in mice

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**AIM:** Evaluation of the antimicrobial and regenerative effect of plasma-activated water (PAW) on a murine model of skin wounds infected with *Staphylococcus aureus* in CD1 mice.

**BACKGROUND:** Wound healing is a complex and dynamic process that can be affected by various factors including infection. PAW emerged as a promising antimicrobial agent and studies are necessary to evaluate its *in vivo* efficacy.

**METHOD:** Eighteen female CD1 mice were divided into two groups: infected with MRSA ATCC 43300 and treated with saline solution (control), infected and treated with plasma-activated water (PAW). An 8 mm skin wound model with anti-contraction silicone rings was created. A volume of 50  $\mu$ L inoculum containing  $10^7$  CFU MRSA was used to infect the wounds. At day 7, 12 and 25 post-infection, immunohistochemical analysis of collagen types 1, 3, 4, cytokeratin 10, and VEGF along with bacterial load evaluation of each wound were performed.

**RESULTS:** The bacterial burden (CFU/wound) decreased rapidly in PAW group comparing to control group:  $2.01 \times 10^5$  vs.  $1.58 \times 10^5$  (day 7),  $0.07 \times 10^3$  vs.  $5.6 \times 10^3$  (day 12),  $<10^1$  vs.  $9.45 \times 10^3$  (day 25). Wound area reduction was accompanied by significant neoangiogenesis indicated by intense VEGF staining and fibrillar neogenesis highlighted by expression of collagen types 1, 3, and 4. The immunohistochemical staining of cytokeratin 10 was intense, correlating with appropriate wound epithelialization and significantly faster healing for the PAW group (day 13) vs. control group (day 18).

**CONCLUSION:** The obtained results confirm the antimicrobial effect of PAW by significantly reducing the presence of MRSA in experimentally infected wound.

### Hyperimmune egg-based IgY-rich formulations as adjuvant therapy in a murine model of urinary tract infection

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**AIM:** Evaluating the efficacy of two Immunoglobulin Y-rich products using a murine model of *Escherichia coli* urinary tract infection.

**BACKGROUND:** Uropathogenic *Escherichia coli* is responsible for the majority of urinary tract infections (UTIs), with a recurrence rate of 25% within 6 months of treatment.

**METHOD:** Thirty female C57BL/6 mice susceptible to uropathogenic *Escherichia coli* (UPEC) DSM 103538 strain were equally divided into three groups: Control (C), ImmunoinstantG (IG), and Immunoinstant Anti-*Escherichia coli* (IE). A 30  $\mu$ L inoculum of UPEC containing  $5 \times 10^7$  CFU was administered intravesical to induce infection. Each experimental group received a dose/kg equivalent to an adult human weighing 70 kg (0.9 mg IgY/mouse in 0.35 mL) administered via gavage. The control group received the same volume of drinking water. The products were administered daily for 22 days, starting 7 days before infection. Bacterial load in urine was evaluated on days 7, 10, and 15.

**RESULTS:** The results showed a significant reduction ( $p < 0.05$ ) in the number of CFUs of UPEC in the treated groups compared to the control group. On day 7, the bacterial load was  $1.8 \times 10^6$  CFU/mL in group C,  $1.9 \times 10^5$  CFU/mL in group IG, and  $1.6 \times 10^5$  CFU/mL in group IA, while on day 15 the bacterial load was  $3.2 \times 10^4$  CFU/mL,  $6.0 \times 10^3$  CFU/mL, and  $4.2 \times 10^3$  CFU/mL respectively.

**CONCLUSION:** The tested products significantly reduced bacterial load with no notable differences between them, suggesting both could be effective alternatives for preventing and treating UTIs.

## Outcomes of post-traumatic osteomyelitis in a conflict setting: a retrospective cohort study in Gaza

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**AIM:** Assess the microbiology and treatment outcomes of post-traumatic osteomyelitis (PTO) patients in Medecins Sans Frontieres (MSF) supported reconstructive surgical facilities in Gaza, pre-October 7, 2023, and identify recurrence risk factors.

**BACKGROUND:** PTO is common among war-wounded in conflict-affected settings in the Middle East. The ongoing war in Gaza since October 2023, has severely disrupted healthcare, increasing suspected and sub-optimally treated PTO, and related literature is scarce.

**METHODS:** Two-centre retrospective cohort study including PTO patients diagnosed by microbiological confirmation via bone biopsy and treated between December 6, 2018 and September 8, 2021, with follow-up until January 31, 2022. Differences between multi-drug resistant (MDR) and non-MDR, polymicrobial and monomicrobial PTO were assessed. Predictors of recurrence were identified using cox proportional hazards multivariate regression.

**RESULTS:** 202 patients with 275 PTO episodes and 441 isolates were included. MDR was present in 53% of episodes; 43% episodes were polymicrobial; recurrence occurred in 26%. Twenty patients (10%) underwent amputation. *Staphylococcus aureus* was the most prevalent (35%) isolate (62% methicillin-resistant), followed by 13% Enterobacterales (59% extended-spectrum beta-lactamase producers), 10% *Pseudomonas aeruginosa* and 3% Acinetobacter species. The 6-month survival (recurrence-free) probability was 79% (95% CI: 73-86) decreasing to 56% (95% CI: 47 - 68) by 24 months. Significant risk factors of recurrence included up to 3 procedures, fibula fractures, PTO with *Enterobacter cloacae* or *Staphylococcus aureus*.

**CONCLUSIONS:** Managing PTO in Gaza is complex. Rebuilding the healthcare system, strengthening local capacities, ensuring access to necessary resources are essential for the long-term management of PTO in Gaza.

## Epidemiological and clinical features of patients with *Clostridoides difficile* infection

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**AIM:** Investigation and identification of epidemiological characteristics, risk factors and treatment regimens effectivity in reducing morbidity and mortality.

**BACKGROUND:** *C.difficile* remains the leading cause of health care associated diarrhea, usually as a result of irrational use of antimicrobial therapy.

**METHODS:** Retrospective descriptive study (October 2020-january2024) included all patients with clinical symptoms of *Clostridoides difficile* infection (CDI) admitted at our clinic. Diagnosis is based on isolation with coproculture for *C.difficile*, toxin confirmation with immunochromatography, small number confirmed with PCR film array.

**RESULTS:** 594 inpatients with clinical symptoms of CDI were evaluated and diagnosis was confirmed in 44. Average mean age was 58,5 (29-90)years, approximately with no differences on sex representation. Comorbidities and previous hospitalisations were noted in two third of them, and 63% had used antimicrobial drugs, while 34% had history of corticosteroid usage and 4,5% immunosuppressive therapy. Often used antibiotics cephalosporins, clindamycine, quinolones and macrolides. 68,2% had history of protein pump inhibitors usage. Culture positive were 38%, toxinA/B is confirmed with immunochromatography in 77% of patients, PCR film array confirmed *C.difficile* toxin A/B in the remaining patients. Treatment is carried out with oral vancomycine in 56% of patients, 15% with metronidazole and the remaining with combination of two drugs. Regarding outcomes 90% of patients were cured while 10% had fatal outcomes and CDI is not considered the main cause of death.

**CONCLUSION:** Elderly patients with antibiotic history treatment, previous healthcare exposures and comorbidities were the most affected by CDI infection. Metronidazole and vancomycine has shown good therapeutic results.

## Impact of Pre-Existing Comorbidities and Multimorbidities, Demography and Viral Variants on Post-Acute Sequelae of COVID-19 ('Long COVID') in Dutch Primary Care: A Retrospective Cohort Study

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**INTRODUCTION:** Post-acute sequelae of COVID-19 (PASC), or 'Long COVID,' involves persistent symptoms following an acute COVID-19 infection, significantly affecting global public health. Despite extensive research, predictors and risk factors of PASC remain under-explored, necessitating comprehensive research based on objective clinical findings. This study examines PASC prevalence, associated comorbidities, demographics, and viral variants using clinical assessment of electronic healthcare records (EHR) from Dutch primary care physicians.

**METHODS:** This retrospective cohort study utilised EHR data from 59 GP practices in northern Netherlands, encompassing 265,000 patients. Included were 19,638 patients who tested positive for COVID-19 between January 1, 2020, and December 31, 2021. PASC was identified using WHO and CDC guidelines, a Dutch pre-trained Word2Vec model, and manual clinical assessment by medical professionals. Patients were categorised based on symptom duration. The impact of pre-existing comorbidities, demographics, and viral variants on PASC was analysed using relative risk (RR) calculations and statistical tests.

**RESULTS:** PASC prevalence was 5.8% (CI95%: 5.4-6.1%) among patients seeking primary care. Key comorbidities increasing PASC risk with statistical significance included lung disease (RR: 1.95), cardiovascular disease (RR: 1.73), diabetes (RR: 1.82), kidney disease