Results. Among the atypical isolates examined, the study identified a number of rare pathogens, including:

- *Elizabethkingia meningoseptica* – a Gram-negative non-fermenting rod associated with nosocomial infections (meningitis in neonates, bacteremia in immunocompromised patients), characterized by intrinsic resistance to multiple antibiotic classes (isolated from a patient's blood).
- *Chryseobacterium indologenes* – an opportunistic non-fermenting microorganism causing infections in intensive care unit patients associated with catheters and mechanical ventilation (isolated from a patient's sputum).
- *Ochrobactrum anthropi* – a rare opportunistic pathogen of catheter-associated bacteremia, difficult to differentiate by conventional methods owing to phenotypic similarity to *Brucella* species (isolated from a patient's sputum).
- *Myroides odoratimimus* – a non-fermenting gram-negative rod with intrinsic multidrug resistance, causing wound and urinary tract infections predominantly in debilitated individuals (isolated from a wound in a military serviceman).
- *Sphingomonas paucimobilis* – a rare pathogen detected in catheter-associated infections and in immunosuppressed patients (isolated from a patient's sputum).
- *Kocuria kristinae* – gram-positive cocci of the family *Micrococcaceae*, with isolated cases described as a cause of bacteremia and endocarditis (isolated from a patient's urine).

Identification of the above microorganisms by conventional culture-based and biochemical methods is frequently complicated by their phenotypic similarity to more common species, which leads to misclassification or a considerable increase in the time required to establish the etiology of the pathological process. The use of MALDI-TOF enabled accurate and maximally rapid verification of all isolates listed.

Conclusions. MALDI-TOF mass spectrometry is a highly effective tool for the rapid identification of rare and atypical pathogens of infectious diseases that are verified with considerable difficulty by conventional methods. Timely and accurate identification of such microorganisms is of fundamental importance for selecting appropriate antimicrobial therapy, epidemiological surveillance, and preventing the spread of healthcare-associated infections of various profiles within clinical settings.

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EFFECT OF THE BACTERIOPHAGE PREPARATION PYOFAG ON PHAGOCYTIC ACTIVITY AND POLARIZATION-ASSOCIATED MARKER EXPRESSION IN RAW264.7 MACROPHAGES

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Abstract. This study evaluated the effects of the polyvalent bacteriophage preparation Pyofag on phagocytic activity and polarization-associated marker expression in RAW264.7 macrophages. Pyofag did not significantly alter the proportion of phagocytic cells, although bead-associated mean fluorescence intensity (MFI) showed a non-significant upward trend. The CD80-positive fraction remained

unchanged, while CD80 MFI tended to increase; CD206 MFI increased 1.6-fold versus the vehicle control ($p = 0.0003$). These findings indicate preserved phagocytic function and a mixed polarization-associated response dominated by enhanced CD206 expression.
Keywords: bacteriophages, Pyofag, RAW264.7, phagocytosis, macrophage polarization.

Background. Antimicrobial resistance is a major global public health threat, driving interest in bacteriophage therapy as a promising alternative or adjunct to conventional antimicrobial treatment (Cieślik et al., 2026). In vivo, phages interact with innate immune cells, and macrophages can contribute to therapeutic phage uptake and clearance, potentially affecting phage persistence (Zborowsky et al., 2025). Macrophage activation is highly plastic and spans a spectrum from predominantly pro-inflammatory and antimicrobial to anti-inflammatory and tissue-reparative states, commonly referred to as M1-like and M2-like phenotypes. Phagocytic function is also important during bacterial infection, as macrophages clear bacteria, dead cells, and tissue debris, contributing to immune defense and tissue homeostasis (Cieślik et al., 2026). Bacteriophages can exert complex, context-dependent effects on macrophages: gut-derived virulent phages induced initial M1-like activation and enhanced phagocytosis followed by tolerance to LPS, whereas M13 phage-coated surfaces promoted an anti-inflammatory M2-like phenotype and enhanced efferocytosis (Hou et al., 2025; Safari et al., 2022). These contrasting effects indicate that macrophage responses to bacteriophages are context-dependent and may extend beyond a simple pro-inflammatory/anti-inflammatory dichotomy. The aim of this study was to evaluate the effect of the polyvalent bacteriophage preparation Pyofag on phagocytic activity and CD80/CD206 expression in RAW264.7 macrophages in vitro.

Materials and methods. RAW264.7 murine macrophages ($5 \cdot 10^5$ cells/sample) were exposed to Pyofag, a polyvalent bacteriophage mixture, at an approximate cell:phage ratio of 1:2 for 30 min at 37 °C, 5 % CO_2. LPS (1 μg/mL) was used as a pro-inflammatory control, while the corresponding phage-free formulation solution served as the vehicle control. After exposure, cells were pelleted, reseeded in complete medium based on high-glucose DMEM, and cultured for 24 h under the same conditions. Phagocytic activity was assessed by incubating cells with 1-μm fluorescent microspheres at a cell:bead ratio of 1:100 for 1 h, followed by washing and flow cytometric analysis. RAW264.7 cells were gated based on FSC/SSC characteristics with subsequent singlet discrimination. Phagocytic activity was quantified as the percentage of bead-positive cells and bead-associated mean fluorescence intensity (MFI), used as an indicator of relative particle uptake. CD80 and CD206 expression was analyzed in separate samples using FITC-conjugated anti-mouse CD80 and PE-conjugated anti-mouse CD206 antibodies. Cells were detached and stained according to the manufacturers' instructions for 30 min at 4 °C, washed, and analyzed by flow cytometry. Marker expression was evaluated as the percentage of positive cells and MFI. Statistical analysis was performed in GraphPad Prism 9.0.0 ($n = 3$ biological replicates; $p \leq 0.05$).

Results. Pyofag did not significantly affect the proportion of phagocytic RAW264.7 cells, which remained high across all groups. Bead-associated MFI tended to increase after Pyofag exposure ($1.26 \cdot 10^6$ vs $0.95 \cdot 10^6$ in the vehicle control) and was comparable to that observed after LPS stimulation ($1.24 \cdot 10^6$), although the difference was not statistically significant. This tendency is consistent with previous observations of enhanced particle phagocytosis following bacteriophage exposure in macrophage models (Hou et al., 2025). LPS significantly increased the proportion of CD80-positive cells compared with the vehicle control ($p = 0.0211$), whereas Pyofag did not significantly alter the CD80-positive fraction; however, CD80 MFI showed a non-significant upward trend. The proportion of CD206-positive cells remained unchanged; however, CD206 MFI increased 1.6-fold after Pyofag exposure compared with the vehicle control ($p = 0.0003$) and was also significantly higher

than after LPS stimulation ($p = 0.0357$). The significant increase in CD206 MFI, together with the non-significant upward trend in CD80 MFI, suggests that the response to Pyofag may involve a mixed pattern of polarization-associated marker expression rather than a unidirectional M1- or M2-like shift. Given its polyvalent composition, the response to Pyofag should be considered a composite effect of multiple phages.

Conclusions. The pattern of polarized activation of RAW264.7 macrophages following exposure to Pyofag does not conform to the classical M1/M2 dichotomous paradigm. A moderate increase in phagocytic activity and a pronounced upregulation of CD206, indicative of an anti-inflammatory metabolic shift, accompanied by a trend toward increased CD80 expression, suggest a complex and multidirectional modulation of macrophage functional state rather than a unidirectional polarization. These findings support the view that Pyofag may induce a mixed or intermediate macrophage activation phenotype. A more precise characterization of this response will require a broader functional assessment, including cytokine production, reactive oxygen species generation, and arginine metabolism.

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SELENIUM NANOPARTICLES SYNTHESIZED USING *LACTOBACILLUS PLANTARUM* IMV B-7679 AND SYNERGISTIC EFFECT OF SELENIUM NANOPARTICLES-ANTIBIOTIC COMBINATIONS

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Abstract. Selenium nanoparticles (Nano-Se) were obtained by using *Lactobacillus plantarum* IMV B-7679. In vitro studies have shown that Nano-Se possess antimicrobial properties and exhibited significant synergistic effects when Nano-Se were combined with traditional antibiotics. The results of this study confirmed that nanoselen could be a potential carrier system for cefotaxime targeting