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# FLOW CYTOMETRY AS A RAPID TEST FOR DETECTION OF TETRACYCLINE RESISTANCE DIRECTLY IN BACTERIAL CELLS IN *MICROCOCCUS LUTEUS*

*Elena Zayko, Dagmara Bataeva, Yuliya Yushina, Anzhelika Makhova, Mariya Grudistova*

## ABSTRACT

Correct effective doses of antibiotics are important in the treatment of infectious diseases. The most frequently used methods for determination of the antibiotic susceptibility of bacterial pathogens are slow. The detection of multidrug-resistant bacteria currently relies on primary isolation followed by phenotypic detection of antibiotic resistance by measuring bacterial growth in the presence of the antibiotic being tested. The basic requirements for methods of detection of resistance to antibiotics include speed and accuracy. We studied the speed and accuracy of flow cytometry for the detection of tetracycline resistance in the Gram-positive bacteria *Micrococcus luteus*. Detection of cell viability and reliability of antibiotic resistance was carried out on the Guava EasyCyte flow cytometer (Merck Millipore, Germany) with SYBR Green and PI dyes. *M. luteus* was exposed to tetracycline (at 30, 90, 180 and 270 μg.mL$^{-1}$) over 24 hours. Concentrations of live and dead cells were measured after 4 and 24 hours of incubation. The results revealed that the use of mixed dyes PI and SYBR Green allowed the division of cells into large subpopulations of live and dead cells and the DNA of destroyed cells. After 4 h exposure to tetracycline 30 μg.mL$^{-1}$, the subpopulation of live cells decreased by 47% compared to the positive control. Tetracycline at 90 μg.mL$^{-1}$ decreased the subpopulation of live cells by 59% compared to the positive control. A continued increase in concentration caused a shift in the population and an increase in dead cells, indicating damage to the cells of the microorganism. Incubation of M. *luteus* with 180 and 270 μg.mL$^{-1}$ tetracycline decreased the subpopulation of live cells by 82% and 94%, respectively, in comparison with the positive control. After incubation with 30 μg of tetracycline over 24 h the number of living cells decreased by 70% in comparison with the positive control. Tetracycline treatment (90 μg.mL$^{-1}$ for 24 h) killed 71% of cells. After exposure to 90 μg.mL$^{-1}$ tetracycline 29% cells were viable. The viability of living cells was confirmed by a microbiological test.

**Keywords:** antibiotics; flow cytometry; antibiotic resistance; Micrococcus luteus

## INTRODUCTION

Antibiotics are one of the most beneficial discoveries in medicine and public health. However, the use, overuse and misuse of these drugs have led to increases in antibiotic-resistant bacterial infections. Antimicrobial resistance (AMR) poses a serious global threat of growing concern to human, animal and environmental health. This is due to the emergence, spread, and persistence of multidrug-resistant bacteria **(Davies and Davies, 2010)**. The O'Neill Review on Antimicrobial Resistance estimated that 700,000 people die from infections due to resistant organisms every year, and by 2050 AMR will surpass cancer as a cause of death **(O'Neill, 2016)**. Resistance to so-called critically important antibiotics used in medicine is of special concern **(Bataeva and Zaiko, 2016)**.

Fast, accurate antibiotic susceptibility testing is a critical need in addressing escalating antibiotic resistance, since delays in identifying multidrug-resistant organisms increase mortality **(Kadri et al., 2018)**.

Standard methods of detection of antibiotic sensitivity are labor- and time-consuming. Detection of multidrug-resistant bacteria currently relies on primary isolation followed by the phenotypic detection of antibiotic resistance by measuring bacterial growth in the presence of the antibiotic being tested. These conventional methods take a minimum of 24 hours to obtain results after the pure culture is isolated (the analysis typically lasts up to 72 hours) **(Akhmaltdinova, Lavrinenko and Belyayev, 2017)**. Working out express diagnostic methods is of importance, and currently, studies are made in various directions **(Wang et al., 2010)**.

One of these directions is the use of flow cytometry (FC) for the detection of microorganism viability and resistance. Flow cytometry was adopted for microbiological purposes almost 40 years ago, and the usefulness of this method for

the identification of microbial pathogens directly in clinical samples or the detection of specific antibodies in serum has been well studied **(Álvarez-Barrientos et al., 2000)**, as has its use in the study of antimicrobial activity of some animal-generated antimicrobial substances **(Kotenkova and Polishchuk, 2019)**.

The quantitative assessment of prokaryotic viability is essential, especially for the confirmation of the activity of novel antimicrobial substances **(Kotenkova et al., 2019)**. Flow cytometry can be used for the analysis of the individual population of cells; therefore, it can provide essential information about bacterial antibiotic resistance. Recently, investigations of bacterial antibiotic resistance seem to be most relevant in the clinical environment because of the problems in finding effective therapies. In comparison with traditional diagnostic methods, FC allows results to be obtained much more quickly **(Álvarez-Barrientos et al., 2000)**. Currently, there are reported experiences of antimicrobial susceptibility tests by FC **(Woźniak-Kosek and Kawiak, 2005; Faria-Ramos et al., 2013)**, but despite the significant progress of clinically significant protocols of FC application, there is insufficient scientific information on its continuous use in microbiology.

This manuscript reports the results of the use of FC with the dyes SYBR Green and PI for rapid assessment of cell viability and antibiotic resistance of the Gram-positive bacteria *Micrococcus luteus*.

## Scientific hypothesis

There is a considerable need for new techniques that enable quick and specific diagnosis of pathogens resistant to antibiotics to guide correct treatment and to slow the development of resistance. Flow cytometry can provide quick essential information about the resistance to antibiotics of pathogenic microorganisms.

## MATERIAL AND METHODOLOGY

*Micrococcus luteus* ATCC 4698 from American Type Culture Collection was used.

## Sample preparation

### *Positive, negative and mixed controls of Micrococcus luteus ATCC 4698*

*Micrococcus luteus* ATCC 4698 strain was obtained from the State Research Center for Applied Biotechnology and Microbiology (Obolensk, Moscow region, Russia). Cultures were grown on slanted Trypticase soy agar (TSA, Liofilchem) at 30 °C for 24 h. Cultures from the agar surface were removed with Trypticase soy broth (TSB, Liofilchem) and incubated at 30 °C for 24 h. Cultures were grown to exponential phase ($OD_{600}$ 0.3). The received suspension was used as a positive control.

To obtain negative control, 1 mL suspension was heated at 100 °C for 10 min. To prepare mixed samples, 200 µL of positive control (live cells) and 200 µL of a negative control (dead cells killed by heating) were used.

### *Preparation of antibiotics*

Cultures were grown to early exponential phase, at which time tetracycline (Sigma-Aldrich, USA) was added in various concentrations (30 $\mu g.mL^{-1}$, 90 $\mu g.mL^{-1}$, 180 $\mu g.mL^{-1}$ and 240 $\mu g.mL^{-1}$). Incubation was carried out at 37 °C for 24 h. Concentrations of live and dead cells were measured after 4 and 24 h of incubation with the antibiotic.

### Flow cytometry analysis protocol

SYBR Green stock solution was prepared by dissolving 5 µL of SYBR ™ Green I Nucleic Acid Gel Stain, 10,000 X concentrate in DMSO (Thermo, USA) in 495 µL of deionized water. A PI working solution was prepared by dissolving 1 mg PI (AppliChem, Germany) in 1 mL of deionized water immediately before the study.

A volume of 975 µL of 1X pH 7.4 PBS (Santa Cruz, USA) was mixed with 10 µL of SYBR Green (Thermo, USA) and 5 µL PI (AppliChem, Germany), shaken vigorously, then 10 µL of *M. luteus* ATCC 4698 was added and mixed. The samples were incubated in the dark for 15 min and green and red fluorescence signals measured on a Guava EasyCyte flow cytometer (Merck Millipore, Germany) for up to 5000 events.

### Microbiology test

Verification of cell viability was obtained by microbiological test by transferring 100 µL of each concentration onto a nutrient agar plate (TSA, Liofilchem). All plates were incubated for 3 – 5 days at 30 °C. The growth on nutrient agar was evaluated by the presence of viable cells in the sample.

### Statistical analysis

Measurements were repeated three times. STATISTICA 10.0 software was used for statistical analyses. The results were calculated as mean ± standard deviation (M ±*SD*). Significant differences (comparison with positive control) were tested by one-way ANOVA *Dunnet* test. Differences with *p*-values less than 0.05 were considered as statistically significant.

## RESULTS AND DISCUSSION

Routine techniques for the detection of resistance to antibiotics are based on a phenotypic study in which microbial growth is observed in the presence of different antibiotics. They yield results in not less than 24 h. In the last two decades, faster AST methods, such as PCR-based tests **(Barken et al., 2007)** and mass spectrometry-based methods **(Opota et al., 2015; Trip et al., 2015)** have been developed. However, they do not always provide relevant information on antibiotic susceptibility. For example, PCR-based tests rely on the detection of resistance mutations and genes. However, bacteria lacking resistance mutations and genes may still be able to tolerate and survive antibiotic treatments by utilizing many other mechanisms, some of which are non-genetic **(Javid et al., 2014; Sanchez-Romero and Casadesus, 2014)**. Changes in bacterial physiology caused by antibiotics can be detected using FC and fluorescent viability markers, as has been demonstrated by numerous studies **(Gant et al., 1993; Walberg et al., 1997; Gauthier et al., 2002; Ambriz-Avina et al., 2014)**.

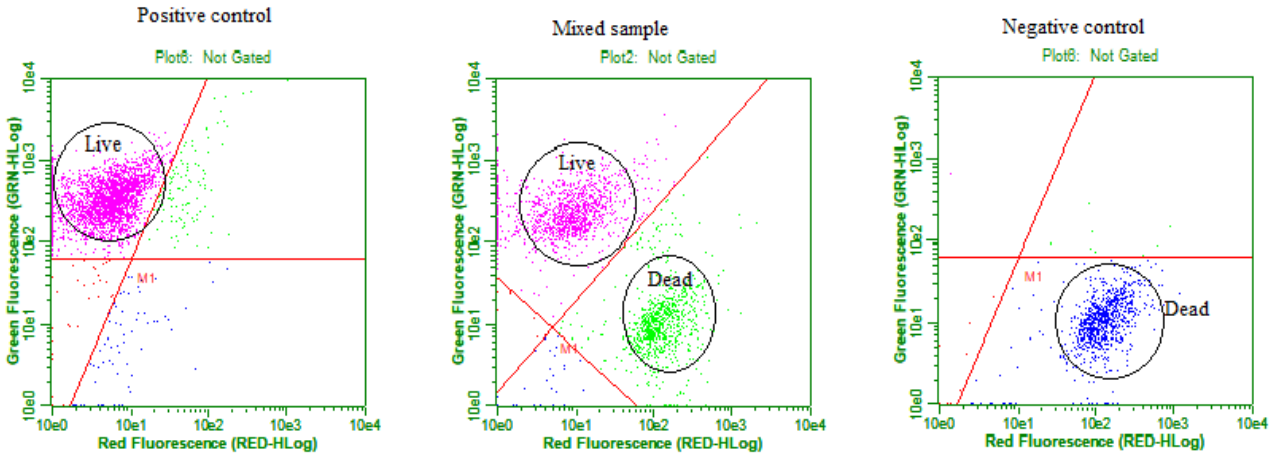


**Figure 1** Result of flow cytometry analysis of positive, negative controls and mixed sample of *Micrococcus luteus* ATCC 4698 without antibotic influence .

*M. luteus* was the object of this study. It is a Gram-positive microorganism with cell walls consisting of two polymers, i.e., peptidoglycans **(Schleifer and Kandler, 1967)** and teichuronic acids (TUAs) **(Hase and Matsushima, 1972)**.

*M. luteus* can form dormant structures that allow for increased survival under stress. So, cold (4 °C), dryness (2.5% humidity) and starvation increase the survival of *M. luteus* **(Casida, 1980)**, possibly by creating unfavorable growth conditions, and thereby inducing dormancy **(Dib et al., 2013)**. Thus, cells can survive long periods under adverse environmental conditions **(Kaprelyants and Kell, 1993)**, which can allow the evaluation of the gradual transition of cells from living to die over time. PI and SYBR Green dyes can identify stained cells in different large subpopulations. Resistant organisms are more difficult to kill than susceptible organisms, and the SYBR Green/PI assay can distinguish resistant and susceptible categories based on the number of residual viable cells after drug treatment **(Feng et al., 2014)**.

Figure 1 presents the staining patterns of *M. luteus* control samples. Positive control *M. luteus* ATCC 4698 (live cells) displayed green fluorescence, and the corresponding counted events are located in the upper left square of the plot. Negative control (dead cells) displayed red fluorescence, and all counted events are located in the lower right square of the plot.

After 4 h exposure of *M. luteus* to tetracycline (at 30 μg.mL$^{-1}$, 90 μg.mL$^{-1}$, 180 μg.mL$^{-1}$ and 270 μg.mL$^{-1}$) the mix of dyes identified the emergence of large subpopulations of live and dead cells and the DNA of destroyed cells (Figure 2).

In Figure 2 the red marker corresponds to live cells, blue to dead cells, and purple to the DNA of destroyed cells. There is an intensification in the displacement of the population of living cells beyond the red marker towards the blue, which indicates dead cells. Since PI and SYBR Green are DNA-binding dyes, it is possible to see a third population on the cytogram – the DNA of destroyed cells. The increase in the number of cells inside the blue marker shows a rapid decline in the numbers of viable bacteria after the addition of antibiotics.

Results of the FC analysis of *M. luteus* after 24 h incubation with various concentrations of tetracycline are presented in Figure 3. The difference in relative mean fluorescence intensity between live and dead cells increased further following 24 h of treatment. A further decrease in the number of living cells from the initially treated cells was noted, and the presence of a large amount of debris indicated that cell lysis was occurring.

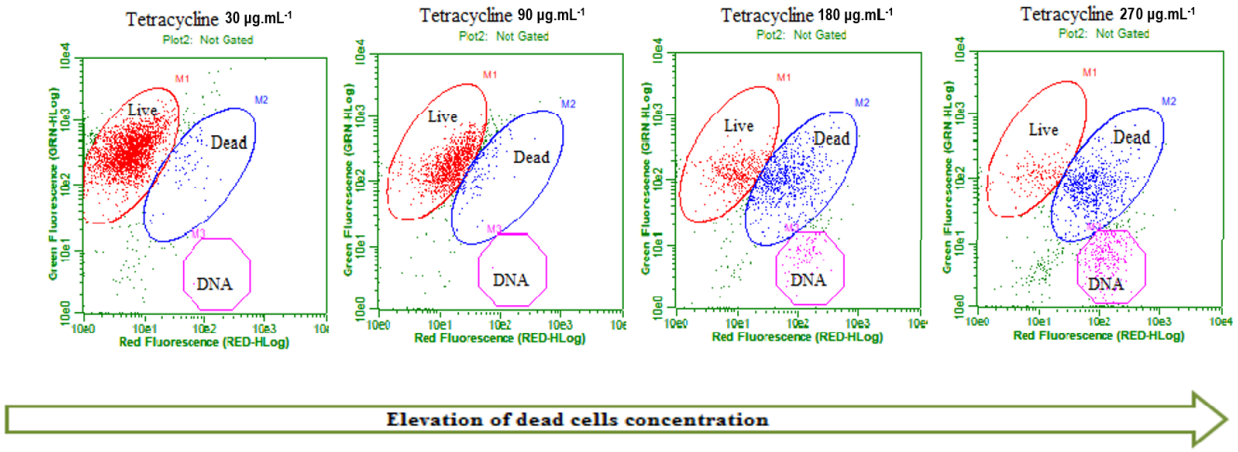


**Figure 2** Results of flow cytometry after exposure of *M. luteus* to tetracycline (at 30 μg.mL$^{-1}$, 90 μg.mL$^{-1}$, 180 μg.mL$^{-1}$ and 270 μg.mL$^{-1}$) during 4 h incubation.

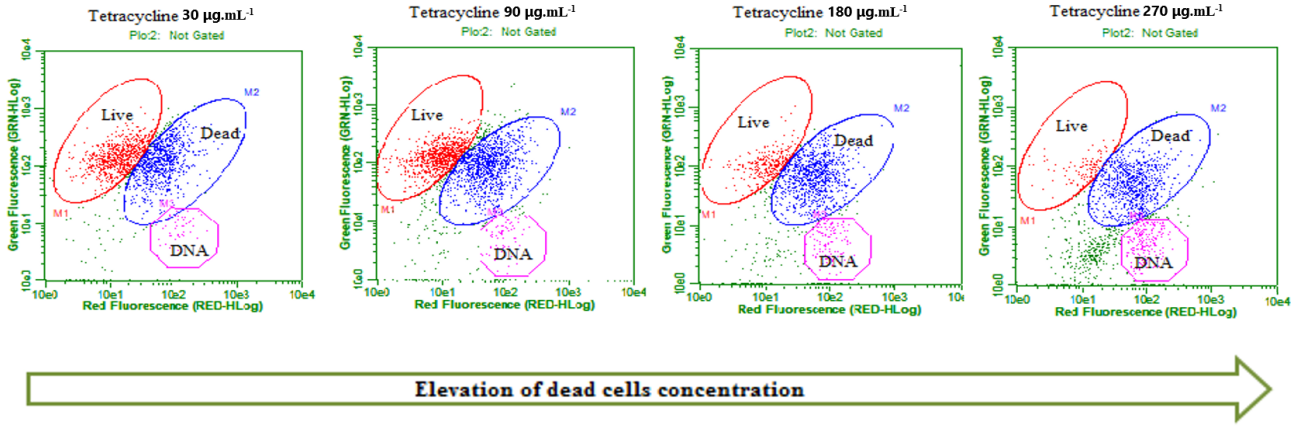


**Figure 3** Results of flow cytometry after exposure of *M. luteus* to tetracycline (at 30 µg.mL$^{-1}$, 90 µg.mL$^{-1}$, 180 µg.mL$^{-1}$ and 270 µg.mL$^{-1}$) during 24 h incubation.

After 4 h exposure to tetracycline at 30 µg.mL$^{-1}$, the subpopulation live cells decreased by 47.0% compared to the positive control (Table 1). Tetracycline solution at 90 µg.mL$^{-1}$ decreased the subpopulation of live cells by 59.0% compared to the positive control.

A continued increase in concentration caused a shift in the population and an increase in dead cells, indicating damage to the cells of the microorganism. So, incubation of *M. luteus* with 180 µg.mL$^{-1}$ and 270 µg.mL$^{-1}$ tetracycline decreased the subpopulation of live cells by 82.0% and 94%, respectively, in comparison with the positive control. The viability of living cells was confirmed by a microbiological test at all concentrations of the antibiotic.

This completely coincided with the results of the cytometric analysis. In analogical research, FC was used for detecting resistant *E. coli* strains. Cytometry showed acceptable results on the model of *E. coli*. Relative accuracy was 88.8%, sensitivity – 85.7%, specificity 88.8%, and Cohen's kappa test showed a value of 0.524 **(Akhmaltdinova, Lavrinenko and Belyayev, 2017)**.

Cytometry analysis showed a decline in the numbers of viable bacteria after 24 h incubation of *M. luteus* with antibiotics. Incubation with 30 µg of tetracycline decreased the number of stained living cells by 70% in comparison with the positive control. Tetracycline treatment at 90 µg.mL$^{-1}$ for 24 h killed 71.0% of cells. So, after exposure to 90 µg.mL$^{-1}$ tetracycline, 29.0% of cells were vaible.

The viability of living cells was confirmed by a microbiological test. Incubation on the plate was increased to 5 days. A study by **Mukamolova et al. (1998)** reported that the viability of *M. luteus* cells was restored after some 96 h incubation of starved cells in a resuscitation medium.

After 24 h exposure to tetracycline (180 µg.mL$^{-1}$ and 270 µg.mL$^{-1}$) the majority of the cells in the population had received antibiotic-induced damage. The number of stained living cells decreased by 89% and 98.9%, respectively, in comparison with the positive control **(Mukamolova, Kaprelyants and Kell, 1998)**. The viability of the remaining living cells was confirmed only at a tetracycline concentration of 180 µg.mL$^{-1}$. Such cell survival can be explained by the ability to transition to cell dormancy in unfavourable growth conditions **(Dib et al. 2013)**. In the study by **Nikitushkin et al. (2016)**, it was reported that in response to unfavourable growth conditions nonsporulating mycobacteria transform into the dormant state with the concomitant formation of specialized dormant forms characterized by low metabolic activity and resistance to antibiotics. This because *M. luteus* secretes a small protein called Rpf, which has autocrine and paracrine signaling functions and is required for the resuscitation of dormant cells **(Nikitushkin, Demina and Kaprelyants, 2016)**.

Dormancy is a protective state that enables bacteria to survive antibiotics, starvation and the immune system. Dormancy comprises different states, including persistent and viable but nonculturable (VBNC) states that contribute to the spread of bacterial infections **(Mali et al., 2017)**.

Food products are a source of antibiotic-resistant pathogenic bacteria, a way for the transmission of antibiotic-resistant 'food' pathogens through the food chain to humans **(Zaiko et al., 2019)**. There is an ongoing increasing antibiotic resistance crisis and new drugs and antibiotics are urgently needed to combat life-threatening antibiotic-resistant infections **(Feng et al., 2018)**.

**Table 1** The results of measuring the number of cells by flow cytometry.

| Time incubation | K «+» | | | Tetracycline | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 30 µg.mL$^{-1}$ | | | 90 µg.mL$^{-1}$ | | | 180 µg.mL$^{-1}$ | | | 270 µg.mL$^{-1}$ | | |
| | *10$^7$ cells.mL$^{-1}$ | | | | | | | | | | | | | | |
| | total | live | dead | total | live | dead | total | live | dead | total | live | dead | total | live | dead |
| **4 hours** | 5.34$^{a}$ | 5.20 | 0.14 | 3.07$^{b,c}$ | 2.80 | 0.27 | 2.84$^{b}$ | 2.10 | 0.74 | 2.40$^{b}$ | 0.90 | 1.5 | 1.50$^{b,d}$ | 0.30 | 1.20 |
| **24 hours** | 22.28$^{a}$ | 22.00 | 0.28 | 3.30$^{b,c}$ | 1.60 | 1.70 | 3.40$^{b}$ | 1.50 | 1.90 | 2.58$^{b}$ | 0.58 | 2.0 | 2.06$^{b,d}$ | 0.26 | 1.80 |

Note: * – significant differences of experimental (tetracycline) doses compared with positive control ($p$ <0.05).

Fast and accurate antibiotic susceptibility tests can significantly reduce mortality rates and reduce financial costs **(Barenfanger et al., 1999)**. Furthermore, the necessity of rapidly prescribing an initial empirical antimicrobial treatment while waiting for the susceptibility test results from time-consuming standard methods frequently leads to inappropriate treatments **(Ibrahim et al., 2000)**.

As to future work, the same strategy may usefully be applied to other microorganisms (including pathogens in difficult food matrixes and other antibiotics. However, the present work shows that one may indeed expect to be able to determine antibiotic susceptibility by FC methods. This could be a very useful tool in the fight against antimicrobial resistance.

## CONCLUSIONS

The current conventional methods for the determination of minimal inhibitory concentration (MIC) rely on the growth of the test organism in the presence of the antibiotic, which can be time-consuming depending on the growth speed of the organism, ranging from days for fast-growing bacteria to weeks for slow-growing bacteria. We reported a rapid and novel antibiotic susceptibility testing methodology using FC. This study to demonstrate the feasibility of FC used SYBR Green/PI dyes to assay rapidly the viability of cells and to detect reliably the antibiotic resistance of the Gram-positive bacteria *Micrococcus luteus*.

## REFERENCES


Akhmaltdinova, L., Lavrinenko, A., Belyayev, I. 2017. Flow Cytometry in Detecting Resistant E. Coli Strains. *Maced. J. Med. Sci.*, vol. 5, no. 5, p. 592-594. https://doi.org/10.3889/oamjms.2017.104

Álvarez-Barrientos, A., Arroyo, J., Cantón, R., Nombela, C., Sánchez-Pérez, M. 2000. Aplication of flow cytometry to clinical microbiology. *Clin. Microbiol. Rev.*, vol. 13, p.167-195. https://doi.org/10.1128/cmr.13.2.167-195.2000

Ambriz-Avina, V., Contreras-Garduno, A., Pedraza-Reyes, M. 2014. Applications of flow cytometry to characterize bacterial physiological responses. *Biomed Res. Int.* 461941.

Barenfanger, J., Drake, C., Kacich, G. 1999. Clinical and financial benefits of rapid bacterial identification and antimicrobial susceptibility testing. *J. Clin. Microbiol.*, vol. 37, no. 5, p. 1415-1418. https://doi.org/10.1128/JCM.37.5.1415-1418.1999

Barken, B., Haagensen, A., Tolker-Nielsen, T. 2007. Advances in nucleic acid-based diagnostics of bacterial infections. *Clinica Chimica Acta*, vol 384, no. 1-2, p. 1-11. https://doi.org/10.1016/j.cca.2007.07.004

Bataeva, D., Zaiko, E. 2016. Risks associated with the presence of antimicrobial drug residues in meat products and products of animal slaughter. *Theory and Practice of Meat Processing*, vol. 1, no. 3, p. 4-13. https://doi.org/10.21323/2414-438X-2016-1-3-4-13

Casida, Jr., L. E. 1980. Death of Micrococcus luteus in Soil. *Applied and Environmental Microbiology*, vol. 39, no. 5 p. 1031-1034. https://doi.org/10.1128/AEM.39.5.1031-1034.1980

Davies, J., Davies, D. 2010. Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Reviews*, vol. 74, no. 3, p. 417-433. https://doi.org/10.1128/MMBR.00016-10

Dib, R., Liebl, W., Wagenknecht, M., Farías, E., Meinhardt, F. 2013. Extrachromosomal genetic elements in Micrococcus. *Appl. Microbiol. Biotechnol.*, vol. 97, no.1, p. 63-75. https://doi.org/10.1007/s00253-012-4539-5

Faria-Ramos, I., Espinar, M., Rocha, R., Santos-Antunes, J., Rodrigues, A. G., Cantón, R., Pina-Vaz, C. 2013. A novel flow cytometric assay for rapid detection of extended-spectrum beta-lactamases. *Clin Microbiol Infect.* vol. 19, no. 1, p. E8-E15. https://doi.org/10.1111/j.1469-0691.2012.03986.x

Feng, J., Wang, T., Shi, W., Zhang, S., Sullivan, D., Auwaerter, P. G., Zhang, Y. 2014. Identification of novel activity against Borrelia burgdorferi persisters using an FDA approved drug library. *Emerging Microbes & Infections*, vol. 3, no. 7, p. e49. https://doi.org/10.1038/emi.2014.53

Feng, J., Yee, R., Zhang, S., Tian, L., Shi, W., Zhang, W., Zhang, Y. 2018. A Rapid Growth-Independent Antibiotic Resistance Detection Test by SYBR Green/Propidium Iodide Viability Assay. *Frontiers in Medicine*., vol. 5, 11 p. https://doi.org/10.3389/fmed.2018.00127

Gant, A., Warnes, G., Phillips, I., Savidge, F. 1993. The application of flow cytometry to the study of bacterial responses to antibiotics. *J. Med. Microbiol*., vol. 39, no. 2, p. 147-154. https://doi.org/10.1099/00222615-39-2-147

Gauthier, L., Dziak, R., Kramer, D. J., Leishman, D., Song, X., Ho, J., Radovic, M., Bentley, D., Yankulov, K. 2002. The role of the carboxyterminal domain of RNA polymerase II in regulating origins of DNA replication in Saccharomyces cerevisiae. *Genetics*, no. 162, no. 3, p. 1117-1129.

Hase, S., Matsushima, Y. 1972. Structural studies on a glucose-containing polysaccharide obtained from cell walls of Micrococcus lysodeikticus. III. Determination of the structure. *J Biochem.*, vol. 72, no. 5, p.1117-1128. https://doi.org/10.1093/oxfordjournals.jbchem.a129999

Ibrahim, H., Sherman, G., Ward, S., Fraser, J., Kollef, H. 2000. The influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting. CHEST, vol. 118, no. 1, p. 146-155. https://doi.org/10.1378/chest.118.1.146

Javid, B., Sorrentino, F., Toosky, M., Zheng, W., Pinkham, J. T., Jain, N., Pan, M., Deighan, P., Rubin, E. J. 2014. Mycobacterial mistranslation is necessary and sufficient for rifampicin phenotypic resistance. *Proc. Natl. Acad. Sci. U.S.A.*, vol. 111, no. 3, p. 1132-1137. https://doi.org/10.1073/pnas.1317580111

Kadri, S., Adjemian, J., Lai, Y., Spaulding, A., Ricotta, E., Prevots, D. R., Palmore, T. N., Rhee, C., Klompas, M., Dekker, J. P., Powers, J. H., Suffredini, A. F., Hooper, D. C., Fridkin, S., Daneer, R. L., NIH-ARORI. 2018. Difficult-to-treat resistance in gram-negative bacteremia at 173 US hospitals: retrospective cohort analysis of prevalence, predictors, and outcome of resistance to all first-line agents. *Clinical Infectious Diseases*, vol. 67, no 12, p. 1803-1814. https://doi.org/10.1093/cid/ciy378

Kaprelyants, A., Kell, D. 1993. Dormancy in stationary-phase cultures of Micrococcus luteus: Flow cytometric analysis of starvation and resuscitation. *Applied and Environmental Microbiology*, vol 59, no. 10, p.10-3187.

Kotenkova, E. A., Polishchuk, E. 2019. Assessment of antimicrobial potential of substances isolated from some wastes of meat processing industry. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 13, no. 1, p. 308-313. https://doi.org/10.5219/1079

Kotenkova, E., Bataeva, D., Minaev, M., Zaiko, E. 2019. Application of EvaGreen for the assessment of Listeria monocytogenes ATCC 13932 cell viability using flow

cytometry. *AIMS Microbiol.*, vol. 5, no. 1, p.39-47. https://doi.org/10.3934/microbiol.2019.1.39

Mali, S., Mitchell, M., Havis, S., Bodunrin, A., Rangel, J., Olson, G., Widner, W. R., Bark, S. J. 2017. A Proteomic Signature of Dormancy in the Actinobacterium Micrococcus luteus. *J Bacterioli*.i, vol. 199, no. 14, p. e00206-17. https://doi.org/10.1128/JB.00206-17

Mukamolova, G., Kaprelyants, A., Kell, D. 1998. On resuscitation from the dormant state of *Micrococcus luteus*. *Antonie van Leeuwenhoek*, vol. 67, p. 289. https://doi.org/10.1007/BF00873692

Nikitushkin, V., Demina, G., Kaprelyants, A. 2016. Rpf Proteins Are the Factors of Reactivation of the Dormant Forms of Actinobacteria. *Biochemistry (Moscow)*. vol. 81. p.1719-1734. https://doi.org/10.1134/S0006297916130095.

Opota, O., Croxatto, A., Prod'hom, G., Greub, G. 2015. Blood culture-based diagnosis of bacteraemia: state of the art. Clin. *Microbiol. Infect*. vol.21, p. 313–322. https://doi.org/10.1016/j.cmi.2015.01.003

Sanchez-Romero, M. A., Casadesus, J. 2014. Contribution of phenotypic heterogeneity to adaptive antibiotic resistance. *Proc. Natl. Acad. Sci. U.S.A.*, vol. 111, no. 1, p. 355-360. https://doi.org/10.1073/pnas.1316084111

Schleifer, K. H., Kandler, O. 1967. Micrococcus lysodeikticus: a new type of cross-linkage of murein. *Biochemical and Biophysical Research Communications,* vol. 28, no. 6, p. 965-972 https://doi.org/10.1016/0006-291X(67)90074-5

O'Neill, J. 2016. The Review on Antimicrobial Resistance. Tackling drug-resistant infections globally: Final report and recommendations. *HM Government*, p. 1-72.

Trip, H., Mende, K., Majchrzykiewicz-Koehorst, A., Sedee, J., Hulst, G., Jansen, H-J., Murray, C. K., Paauw, A. 2015. Simultaneous identification of multiple beta-lactamases in acinetobacter baumannii in relation to carbapenem and ceftazidime resistance, using liquid chromatography-tandem mass spectrometry. *J. Clin. Microbiol.i,* vol. 53, no. 6, p.1927-1930. https://doi.org/10.1128/JCM.00620-15

Walberg, M., Gaustad, P., Steen, B. 1997. Rapid assessment of ceftazidime, ciprofloxacin, and gentamicin susceptibility in exponentially-growing E. coli cells by means of flow cytometry. *Cytometry*, vol. 27, p. 169-178. https://doi.org/10.1002/(SICI)1097-0320(19970201)27:2<169::AID-CYTO9>3.0.CO;2-B

Wang, Y., Hammes, F., De Roy, K., Verstraete, W., Boon, N. 2010. Past, present and future applications of flow cytometry in aquatic microbiology. *Trends Biotechnol.*, vol. 28 p. 416-24. https://doi.org/10.1016/j.tibtech.2010.04.006

Woźniak-Kosek, A., Kawiak, J. 2005. Flow cytometry analysis of the activity of disinfecting agents tested with Staphylococcus aureus and Escherichia coli. *Pol. J. Microbiol*., vol. 54, no. 1, p. 21-26.

Zaiko, E., Panchenko, A., Satabaeva, D., Bataeva, D. 2019. Prevalence and resistance to antibiotics of pathogenic microorganisms isolated from the meat of various animal species. *Vsyo o myase*, vol. 3, p. 42-45. https://doi.org/10.21323/2071-2499-2019-3-42-45

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**Contact addresses:**
*Elena Zayko, researcher, V. M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences, Department of Hygiene of Production and Microbiology, Talalikhina st. 26, 109316, Moscow, Russia, Tel.:8-960-548-71-95,
E-mail: e.zaiko@fncps.ru
ORCID: https://orcid.org/0000-0002-5048-9321

Dagmara Bataeva, researcher, V. M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences, Department of Hygiene of Production and Microbiology, Talalikhina st. 26, 109316, Moscow, Russia, Tel.:8-985-663-84-06,
E-mail: d.bataeva@fncps.ru
ORCID: https://orcid.org/0000-0002-1374-2746

Yuliya Yushina, head of department, V. M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences, Department of hygiene of production and microbiology, Talalikhinast. 26, 109316, Moscow, Russia, Tel.: 8-916-433-51-99,
E-mail: yshinauk@mail.ru
ORCID: https://orcid.org/0000-0001-9265-5511

Anzhelika Makhova, researcher, V. M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences, Department of Hygiene of Production and Microbiology, Talalikhina st. 26, 109316, Moscow, Russia, Tel.:8-916-570-91-79,
E-mail: a.mahova@fncps.ru
ORCID: https://orcid.org/0000-0002-2508-2888

Mariya Grudistova, researcher, V. M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences, Department of Hygiene of Production and Microbiology, Talalikhina st. 26, 109316, Moscow, Russia, Tel.:8-916-311-64-30,
E-mail: m.grudistova@fncps.ru
ORCID: https://orcid.org/0000-0002-8581-2379

Corresponding author: *