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Flow Cytometry in Detecting Resistant *E. Coli* Strains

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Abstract

Antibacterial drugs are the most consumed group of drugs in the modern hospitals. Standard methods of antibiotic sensitivity are labour and time-consuming, taking up to 24 hours after the pure culture is isolated (the analysis typically lasts up to 72 hours). Working out express diagnostic methods is of importance, and studies are made in various directions. Flow cytometry in detecting resistant E. coli strains was used. Flow cytometry fluorescent dyes were used to stain viable and dead cells. For method validation, relative accuracy, relative susceptibility, relative specificity and Cohen's kappa test were determined compared to the delusion test. Cytometry method showed acceptable results on the model of *E.coli.* Relative accuracy comprised 88.8%, sensitivity - 85.7%, specificity was 88.8%, Cohen's kappa test showed value 0.524, which is a medium agreement between the measurements by different methods.

Introduction

Antibacterial drugs are the most consumed group of drugs in the modern hospitals. Standard methods of antibiotic sensitivity are labour and time-consuming, taking up to 24 hours after the pure culture is isolated (the analysis typically lasts up to 72 hours). Working out express diagnostic methods is of importance, and studies are made in various directions [1].

Flow cytometry is a relatively new, but popular technology, and is used in various clinical spheres, such as Immunology, Oncology, Transplantation, Microbiology, Sea Biology and Industrial Biotechnology. The potential of the analysis of various cells' parameters and that of their morphology constantly expands. So do the functional potential of the analysis automation and acceleration. There are experiences of antimicrobial susceptibility test by flow cytometry [2-5]. But at the moment, despite the significant progress of clinically significant protocols of FC application, there is not enough suggestion in clinical microbiology.

Methods

Clinical strains from patients were used after their identification and check for antibiotic sensitivity with commonly used methods. The absence and presence of significant antibiotic resistance were taken into consideration. 20 *E. coli* strains were used: Ten productive ESBL and ten nonproductive ESBL. The isolation of pure cultures from clinical material was performed according to the commonly accepted scheme [6]. Strain reidentification was done with MALDI-TOF mass spectrometry method. Antibiotic sensitivity was determined using disk diffusion method and serial dilution antibiotic susceptibility testing [6, 7], the latter taken as a gold standard test and the comparison method.

Partec CyFlow cytometer was used. Flow cytometry fluorescent dyes were used to stain viable and dead cells. Propidium iodide (by Thermo Fisher Scientific) (water PI solution with the final concentration of 1 μg/ml, based on the proportion of 5 μl to 100 μl of culture, 5-minute incubation in dark) and SYTO® 9 (by Thermo Fisher Scientific) working

solution (with the final concentration of 1 μM/ml 5 μl per 100 μl of culture, 10-minute incubation) were used. The bacterial culture with culture concentration 0.5 MF was placed into the Muller-Hinton broth where the target antibiotic was present. Cefotaxim 0.2 mg/ml and Amoxicillin clavulanate 0.25 mg/ml were used. Incubation time was 2 hours.

For method validation, relative accuracy (AC), relative sensibility (SE) and relative specificity (SP) were determined compared to the delusion test. Cohen's kappa test, which determines the measure of agreement changing from 0 to 1, at the same time accepting or rejecting H-null, and the limit of confidence, was used to evaluate the methods' agreement. If $\kappa > 0.75$, the agreement between the measurements by different methods is considered high; if $0.65 < \kappa \leq 0.75$, the correlation is good; in case of $0.4 < \kappa \leq 0.65$, it is medium; other figures mean bad agreement.

Results

The average minimal inhibiting concentration of Amoxicillin clavulanate for ESBL + strains was 59.2 ± 16.9 mg/l (the strains offered were either moderately resistant or resistant), but for ESBL – strains, it was 14.4 ± 6.5 mg/l (all strains resistant). The average minimal inhibiting Cefotaxim concentration was 14.4 ± 6.5 mg/l for ESBL + strains (all were resistant), but in the case of ESBL – strains, it was 1.5 ± 0.53 mg/l (all were sensitive).

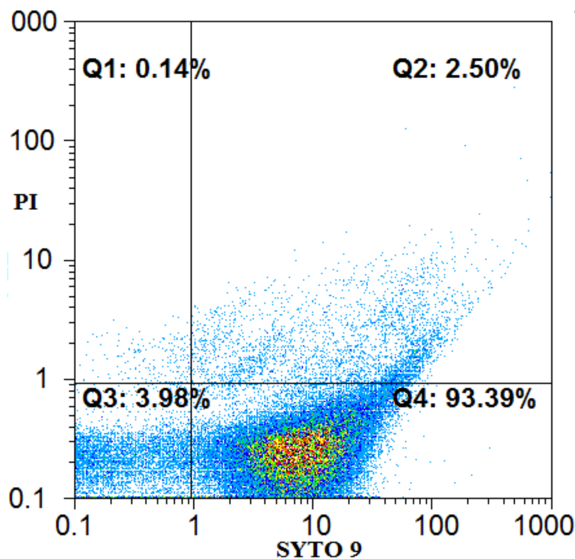

Figure 1: Syto PI-stained E. coli without antibacterial influence

The average PI+ percent calculated for strains invariably sensitive (ESBL-) to Amoxicillin clavulanate comprised 71.3% ± 3.9%, and in the case of Cefotaxim, it was 70.4% ± 4.2%. The average PI+ percent of the population of invariably beta-lactam-resistant strains (ESBL+) was 22.8% ± 5.3% with Amoxicillin clavulanate and 25.0% ± 5.7% with Cefotaxim.

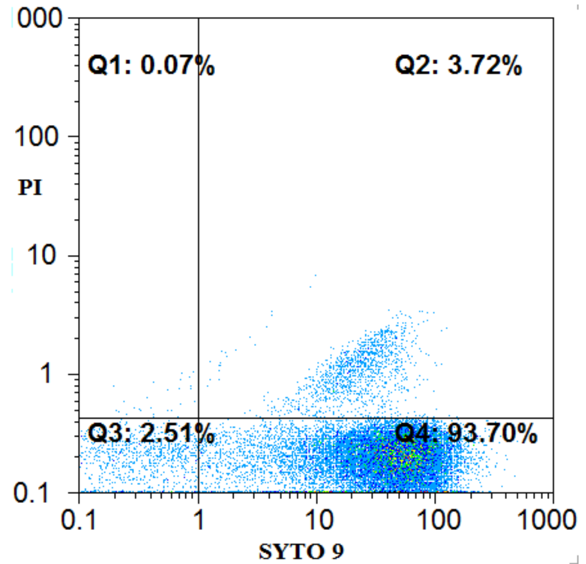

Figure 2: Syto PI – stained resistant E. coli strain

Figure 1 presents SytoPI-stained *E.coli* histogram in the absence of antibiotic drug, PI+ concentration is 2.5%. Figure 2 shows PI+ percent of only 3.72 as a result of strain's resistance to the antibacterial drug. Figure 3 is a diagram showing strain sensitivity to antibacterial action, with 63.8% of the population having the signs of membrane damage after 2- hour incubation.

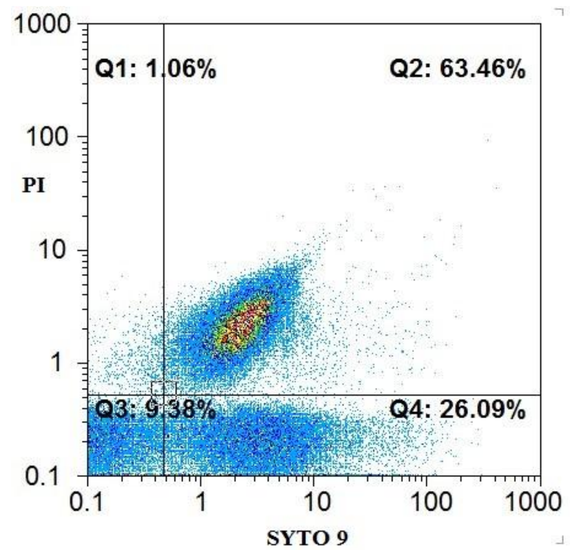

Figure 3: Syto PI-stained strain, sensitive to antimicrobial drugs

On Figure 4, the result for highly- sensitive strain is given, of which 95.85% was PI-stained, and 39.43% was not stained with Syto, so dead cells were determined. On figure 4, the result for highly- sensitive

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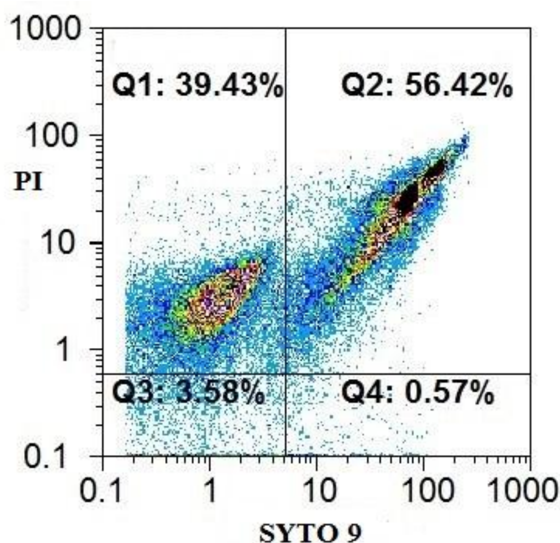

Figure 4: Syto PI-stained E.coli strain, highly sensitive to antimicrobial drugs

Based on the results, a table of pseudo positive and pseudo negative results of cytometry method was built – Table 1.

Table 1: Method Validation based on *E. coli* model

Cytometry Test Result	Sensitivity revealed with standard test (S)	Resistance revealed with standard test (R)
Antibiotic sensitivity (S) revealed	18	3
Antibiotic resistance (R) revealed	3	16

Relative accuracy (AC), relative specificity (SP), relative sensitivity (SE), Cohen's kappa and their limits of confidence were calculated. The measure of agreement value of 0.524 was taken as a medium.

Table 2: Accuracy, sensitivity, specificity and Cohen's kappa of Cytometry method based on E. coli model as compared with classical antibiotic sensitivity determination methods

AC	SP	SE	k
85.0%	85.7	88.8%	0.524
79.4-90.6	77.7-93.7	82-95.6	0.191-0.857

Discussion

Cytometry method showed acceptable results on the model of *E. coli.* Relative accuracy comprised 88.8%, sensitivity - 85.7%, specificity was 88.8%, and agreement test showed value 0.524, which is medium. There were both pseudo positive and pseudo negative results. In general, the method under discussion has shown good agreement with the commonly accepted Gold standard method, delusion test. There was the insignificant difference in accuracy, specificity and test sensitivity revealed, depending on the culture tested. As to the negative aspects, although the tube-test is less time and labour-consuming compared with delusion test, it is still not advantageous in comparison with the disc-diffusing method. However, the reduction of time provided by the method – 2.5 hours from pure culture isolation – is an advantage.

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