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# Intereactions between doripenem and clavulanate — Application of minimal inhibitory concentration analysis and cytometry flow for bactericidal studies

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ABSTRACT

*Background:* In view of the current low efficacy of bacterial infection treatment the common trend towards searching for antibiotic systems exhibiting synergistic action is well justified. Among carbapenem analogues a particularly interesting option is provided by combinations of clavulanic acid with meropenem, which have proven to be especially effective.
*Results:* Determination of the minimal inhibitory concentration (MIC) along with the method based on flow cytometry constitutes an important tool in the identification of bacterial sensitivity to active substances. Within this study the inhibitory effect of doripenem, clavulanic acid and the doripenem-clavulanate acid system was analyzed in relation to such bacteria as *Salmonella enteritidis*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Clostridium butyricum* and *Clostridium pasteurianum*, *Acinetobacter baumannii*, *Enterobacter aerogenes*. The lowest MIC, amounting to 0.03 μg/mL, was observed for the doripenem-clavulanate acid system in the case of *E. coli* ATCC 25922. In turn, the lowest MIC for doripenem applied alone was recorded for *K. pneumoniae* ATCC 31488, for which it was 0.1 μg/mL. The strain which proved to be most resistant both to doripenem and the doripenem-clavulanate acid system, was *A. baumannii*, with MIC of 32 μg/mL (clinical isolate) and 16 μg/mL (reference strain). Cytometric analysis for *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923 showed changes in cells following exposure to limiting concentrations of the active substance.
*Conclusions:* Analysis of MIC supplies important information concerning microbial sensitivity to active substances, mainly in terms of limiting concentrations causing mortality or vitality of the tested species, which is essential when selecting appropriate antibiotic therapy.



## 1. Introduction

Doripenem is a carbapenem (a β-lactam antibiotic) and contains a carboxyl group in the C-2 position and a trans-β-hydroxyethyl group in the C-6 position. Similar to the other carbapenems excluding ertapenem, it is used in the acid form and exhibits pharmacokinetic parameters comparable to those of meropenem and imipenem [1]. The mode of action of doripenem, similarly as in the case of other carbapenems, consists in the inhibition of bacterial cell wall biosynthesis by binding with penicillin binding proteins (PBPs) [2]. Affinity of doripenem to various PBP types depends on microorganisms. In the case of *Escherichia coli* doripenem shows the greatest affinity to PBP2, in *Pseudomonas aeruginosa* it is to PBP3, while in *Staphylococcus aureus* it is to PBP1, PBP2 and PBP4 [2,3]. Among carbapenems the spectrum of microbiological activity of doripenem is closest to that of meropenem [4]. Doripenem was authorized for use by the U.S. Food and Drug Administration (FDA) in 2007 and by the European Medicines Agency (EMA) in 2008 [5,6]. In Europe it was registered as a drug for treatment of adult patients suffering from such infections as hospital acquired pneumonia, including ventilator-associated pneumonia (VAP), complicated infections within the abdominal cavity and complicated infections of the urinary tract, including pyelonephritis.

Resistance of bacteria to β-lactam antibiotics is determined by diverse mechanisms e.g. the production of β-lactamases, enzymes hydrolyzing β-lactam molecules. This mechanism may be eliminated by supplementing the active substance with a respective inhibitor. Clavulanic acid (CA) may be an example of such a compound. CA is the first clinically useful β-lactam inhibitor with a high affinity to class

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A β-lactamase [7,8,9,10] and, in effect, an irreversible inhibitor of intracellular and extracellular β-lactamases. Consequently, combinations of clavulanic acid with penam analogs (amoxicillin) are widely used to treat bacterial infections of various etiology. CA exhibits activity against a broad spectrum of Gram-positive and Gram-negative bacteria. However, in comparison to other antibiotics this activity is relatively low. As a result, CA may not be used as an independent bactericide. The application of CA is based on the formation of its complexes with other broad-spectrum antibiotics susceptible to the action of β-lactamases [11].

In view of the current post-antibiotic era with the observed low efficacy of bacterial infection treatment the common trend towards searching for antibiotic systems exhibiting synergistic action is well justified. Among carbapenem analogues a particularly interesting option is provided by combinations of CA with meropenem, which have proven to be especially effective in treatment of multidrug-resistant *Mycobacterium tuberculosis* [12]. Literature on the subject also presents reports in the combination of meropenem with systems of other chemotherapeutics, e.g. amoxicillin-clavulanate and linezoid-calvulanate combinations [13,14] in order to increase bactericidal response to *M. tuberculosis*. Moreover, we need to mention here a stronger bactericidal response for the meropenem-clavulanate system in relation to bacterial strains posing a clinical problem in therapies targeting *P. aeruginosa, S. aureus* and *L. monocytogenes* [15].

Nowadays, one of the biggest fields of interest is to develop methods which enable fast, accurate assessment of presence of microorganisms and also its viability and functionality [16]. Traditional analytical methods provide information about whole population of microorganisms and there is no possibility to distinguish intermediate states of evaluated objects. In comparison, methods using fluorescent markers allow single-cell analysis and enable distinguish various subpopulations [17,18]. Flow cytometry is an advanced method used for quantitative, as well as qualitative assessment of individual microbial cells. Cytometric measurement can be based both on light dispersion and fluorescence emitted by tested cells. Direct analytical tools of this type don't require growth of microorganisms on artificial media, therefore it gives an opportunity to assess the existence of viable but non-culturable microbial cells [18,19,20].

This method provides an information about cell complexity and physiology, there are many fluorescent probes to evaluate such parameters as membrane potential and integrity, enzyme activity and intracellular pH [16,17]. It is possible to assess few parameters of tested cells at once, using simultaneous staining with more than one fluorescent dye. Multiparametric analysis provides quick and precise information about dynamics and physiological differentiation within the population [20,21]. At first, flow cytometry was developed for different clinical applications, like oncology or hematology. Now use of this technique expands on microbiology field with great results and enables obtaining information about very complex, heterogenous samples in short period of time [16,17].

The aim of this study was to determine the minimal inhibitory concentration for selected indicator microorganisms along with the cytometric analysis illustrating changes in limiting concentrations inhibiting microbial growth for the binary system of doripenem and clavulanic acid, while bactericidal activity of doripenem and clavulanic acid was considered as the reference value.

## 2. Material and methods

### 2.1. Microorganisms

The bacterial strains analyzed using MIC analysis and flow cytometric assay included ATCC reference strains and clinical isolates (*) from the Institute of Laboratory Medicine at the Clinic Poznan, Poland. A total of 22 microorganisms were included in this study. The reference strains were obtained from the American Type Culture Collection (ATCC) and included: *Salmonella enteritidis* ATCC 13076, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* ATCC 31488, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 8427, *Clostridium butyricum* ATCC 860, *Clostridium pasterianum* ATCC 6013, *Acinetobacter baumannii* ATCC 19606, *Enterobacter aerogenes* ATCC 13048.

### 2.2. Microbiological analysis

Minimal Inhibitory Concentration (MIC) was determined for each reference strain from the American Type Culture Collection and clinical isolates. MICs for doripenem, clavulanate acid and their system were assayed using serial dilutions on the Mueller–Hinton liquid medium (Merck, Germany). Microbial culture with a standardized optical density was used in that experiment. The applied method follows the standards of the National Committee for Clinical Laboratory Standards (NCCLS) [22]

### 2.3. Flow cytometry

In the flow cytometric susceptibility assay two bacterial reference strains were investigated, i.e. *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923. Samples were analyzed using a Guava easyCyte™ 8 (Merck Millipore) flow cytometer, equipped with 2 lasers (488 nm and 640 nm), 6 fluorescence detectors, forward scatter (FSC) and side scatter (SSC) detectors. The instrument setup (optical alignment) was performed using the guava® easyCheck Kit (Merck Millipore). The particles were characterized by two non-fluorescent parameters: forward scatter (FSC) and side scatter (SSC), and one fluorescent parameters - red fluorescence from propidium iodide (PI). A 640 nm red laser was employed in excitation of the fluorescent reagent. Forward scatter (FSC) and side scatter (SSC) measurements were applied in the analyses. FSC and SSC parameters are designated to cell size and complexity. Flow cytometry analyses were performed using logarithmic gains and specific detector settings (5000 events were recorded per analysis). The threshold was set at the FSC signals. Data were acquired in a four-decade logarithmic scale as height signals and analyzed with the guavaSoft 2.6 software (Merck Millipore). Samples were analyzed in triplicates.

## 3. Results

The primary aspect of the study comprised analyses of the effect of doripenem and clavulanic acid as well as the system of doripenem with clavulanic acid on eleven selected species of indicator microorganisms. Analyses were performed on reference strains and clinical isolates (Table 1). *C. pasteurianum, K. pneumoniae* and *E. aerogenes* were the microbial species exhibiting the greatest sensitivity to doripenem. The minimal inhibitory concentrations were 0.25, 0.1 and 0.25 μg/mL for reference strains and 0.25, 2 and 1 μg/mL, respectively, for clinical isolates. The highest MIC values were recorded for strains of *A. baumannii* and *S. aureus* both in the case of reference strains and clinical isolates. For *A. baumannii* ATCC 19606 MIC it was 16 μg/mL in relation to doripenem, while for the clinical isolate of that species it was 32 μg/mL. In the case of *S. aureus* MIC was 8 μg/mL both for the reference strain and the clinical isolate. Moreover, the effect of clavulanic acid was investigated for selected microbial species exhibiting a pathogenic potential. No effect of the β-lactamase inhibitor on bacteria from the genus *Salmonella,* or such species as *K. pneumoniae, C. butyricum* and *A. baumannii.* For the other species MIC values were much higher than for doripenem. In the case of *E. aerogenes* MIC for CA was 250 μg/mL, for *P. aeruginosa* MIC amounted to 125 μg/mL, while for *P. vulgaris* it was 32 μg/mL both for reference strains and clinical isolates. A combination of doripenem and clavulanic acid in many cases made it possible to

**Table 1**
In vitro activity of doripenem, clavulanic acid and doripenem-clavulanic acid system.

| Microorganism reference stain or clinical isolates | Doripenem | Clavulanic acid | Doripenem - clavulanic acid system |
|---|---|---|---|
| | μg/mL | | |
| *Salmonella enteritidis*[a] | 2 | No effect | 2 |
| *Salmonella enteritidis* ATCC 13076 | 1 | No effect | 1 |
| *Salmonella typhimurium* | 4 | No effect | 4 |
| *Salmonella typhimurium* ATCC 14028 | 2 | No effect | 2 |
| *Staphylococcus aureus*[a] | 8 | 8 | 4 |
| *Staphylococcus aureus* ATCC 25923 | 8 | 8 | 4 |
| *Klebsiella pneumoniae*[a] | 2 | No effect | 2 |
| *Klebsiella pneumoniae* ATCC 31488 | 0.1 | No effect | 0.1 |
| *Escherichia coli*[a] | 4 | 32 | 1 |
| *Escherichia coli* ATCC 25922 | 0.25 | 16 | 0.03 |
| *Pseudomonas aeruginosa*[a] | 4 | 125 | 1 |
| *Pseudomonas aeruginosa* ATCC 27853 | 1 | 125 | 0.1 |
| *Proteus vulgaris* | 4 | 32 | 1 |
| *Proteus vulgaris* ATCC 8427 | 0.5 | 32 | 0.2 |
| *Clostridium butyricum*[a] | No effect | No effect | No effect |
| *Clostridium butyricum* ATCC 860 | No effect | No effect | No effect |
| *Clostridium pasterianum*[a] | 0.25 | 32 | 0.125 |
| *Clostridium pasterianum* ATCC 6013 | 0.25 | 32 | 0.125 |
| *Acinetobacter baumannii*[a] | 32 | No effect | 32 |
| *Acinetobacter baumannii* ATCC 19606 | 16 | No effect | 16 |
| *Enterobacter aerogenes* | 1 | 250 | 1 |
| *Enterobacter aerogenes* ATCC 13048 | 0.25 | 250 | 0.25 |

[a] Clinical izolates.

reduce MIC. An example in this respect may be *S. aureus* (a clinical isolate), for which MIC decreased from 8 μg/mL to 1 μg/mL. In the case of *E. coli* a decrease in MIC was also observed from 4 to 1 μg/mL for the clinical isolate and from 0.25 μg/mL to 0.03 μg/mL for the reference strain. In the case of *P. aeruginosa* and *P. vulgaris* for clinical strains MIC values decreased from 4 to 1 μg/mL, whereas for the reference strain this reduction of MIC levels was recorded from 1 to 0.1 μg/mL and from 0.5 to 0.2 μg/mL. In turn, for *C. pasteurianum*, both the clinical strain and the reference train, MIC levels decreased from 0.25 to 0.125 μg/mL. For the other tested strains no reduction of MIC values was recorded in the variant, in which doripenem was supplemented with the β-lactamase inhibitor.

The last stage of the study consisted in cytometric analyses for *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923, cultured at limiting concentrations inhibiting bacterial growth. The primary objective of this analysis was to illustrate changes in bacterial cell wall integrity at limiting concentrations of the system of two active substances. Fig. 1n and Fig. 2 present example results of cytometric analyses for selected bacterial species applying the standard staining protocol with propidium iodide (PI), which penetrates into cells with disturbed cell wall integrity. Fig. 1 presents changes in *S. aureus* cell wall integrity. The system concentration of 1 μg/mL had no negative effect on examined cells, with the left-hand plot presenting a population of specimens characterized by low fluorescence (Fig. 1c). However, the concentration of 4 μg/mL changed the physiological status of that bacterial population, resulting in the penetration of PI

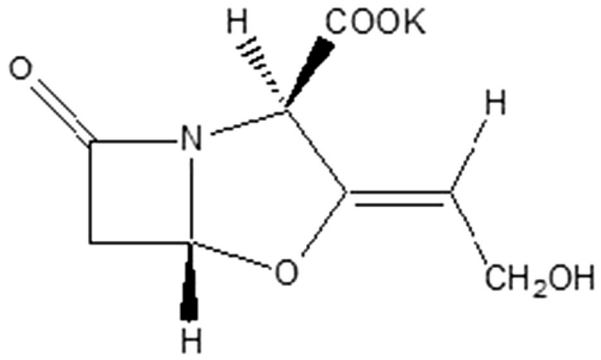


**Fig. 2.** Clavulanic acid.

visualized as an intensive fluorescence signal on the right-hand plot (Fig. 1a). Cytometric analysis for a representative of Gram-negative bacteria, *P. aeruginosa*, showed a difference between Gram-positive and Gram-negative bacteria. Fig. 2 presents changes in cell wall integrity for *P. aeruginosa* ATCC 27853 in the presence of three concentrations of the doripenem-clavulanic acid system, amounting to 0.5, 0.25 and 0.1 μg/mL. The concentration of 0.1 μg/mL had no negative effect on examined cells, on the left-hand plot we may see a population exhibiting low fluorescence (Fig. 2c). However, the concentration of 0.5 μg/mL already drastically changed the physiological status of this bacterial population, resulting in propidium iodide penetration into cells, observed as an intensive fluorescence signal on the right-hand plot (Fig. 2a). (See Fig. 3 and Fig. 4.)

## 4. Discussion

Literature data indicate that doripenem exhibits in vitro activity against intestinal bacteria from the family *Enterobacteriaceae*, including ESBL producers and AmpC-derepression strains. The most extensive described examples include selected strains of *P. aeruginosa*, *Acinetobacter* spp. and *Haemophilus* spp. Results recorded in this study are consistent with literature data and confirm high efficacy of doripenem against *K. pneumoniae*, *E.coli*, *P. vulgaris* and *E. aerogenes*. The highest MIC values were recorded for *Enterobacteriaceae* from the genus *Salmonella*. Doripenem also shows activity against *P. aeruginosa* slightly exceeding that of meropenem and imipenem [4,15]. Similar to the cases of other carbapenems, resistance of specific bacterial strains to doripenem may result from its hydrolysis under the influence of β-lactamases, PBP penetration capacity or activation of the efflux pump. Generally doripenem is insensitive to the action of most β-lactamases; however, it may be hydrolyzed by β-lactamases, Ambler class B such as IMP, SPM or VIM, which may be produced e.g. by *Pseudomonas* spp., *A. baumannii*, *Stenothophomonas maltophilia* or *Bacillus* spp. [23]. The evident resistance of *A. baumannii* may be connected with the activity of these enzyme classes. In turn, reduced permeability of the outer membrane of *P. aeruginosa* to carbapanems may be caused either by a lack or decreased production of the OprD

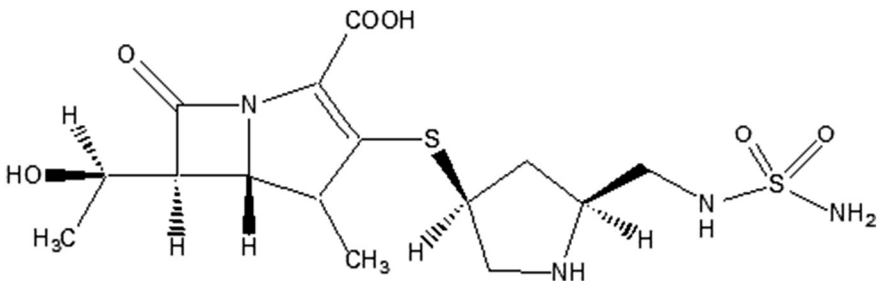


**Fig. 1.** Doripenem.

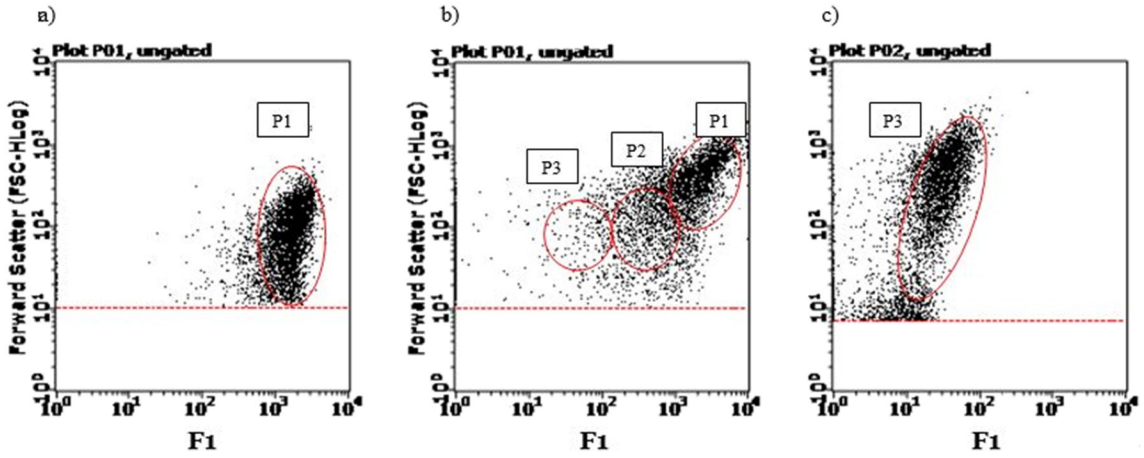


**Fig. 3.** Flow cytometry dot plots of F1 (PI, red fluorescence) of *S. aureus* (ATCC 25923) treated with increasing concentrations of doripenem - clavulanic acid system; (a) 4 μg/mL (MIC); (b) 2 μg/mL; (c) 1 μg/mL. P1: dead cells; P2: cells damaged membranes; P3: live cells.

protein, previously called D2 porin. The primary role of this protein is connected with passive trapping of basic amino acids by the outer membrane. The OprD protein forms pores, which are also permeable for carbapenems, but impermeable for other β-lactam antibiotics. All *P. aeruginosa* strains to a varying degree exhibit natural resistance to these compounds, including interactions of drug impermeability through the outer membrane with the drug efflux involving the MexA-MexB-OprM pump. The efflux system (MexA-MexB-OprM) is composed of proteins: MexB (the pump located in the cytoplasmic membrane), OprM (forming channels in the outer membrane) and MexA binding MexB and OprM proteins. Increased MIC values of penicillins, cephalosporins and quinolones, but not imipenem, are the result of the overactive MexA-MexB-OprM pump caused by the na1B mutation at the mexR locus. These differences are explained by a lack of the heterocyclic substituent in the imipenem molecule. The Mex-A-MexB-OprM overactivity reduces the activity of meropenem at the same time having no effect on resistance to this drug. This indicates that meropenem may use the OprD protein to penetrate to *P. aeruginosa* and that it is recognized and ejected by the MexB system, as it contains a heterocyclic substituent in the chain at C3 of the carbapenem system. Resistance of *P. aeruginosa* to imipenem is mainly determined by the loss of the OprD protein, while resistance to meropenem – by MexA-MexB-OprM over activity. Additionally, a lack or decrease in the production of the OprD protein by *Pseudomonas* spp. contributes to a reduction of MICs for doripenem, similar to the case of imipenem and meropenem [24]. Resistance of *P. aeruginosa* to imipenem is determined mainly by the loss of the OprD protein, while resistance to meropenem and doripenem is also affected by overactivity of MexA-MexB-OprM [24,25]. For this reason *P. aeruginosa* bacteria acquire resistance to meropenem or doripenem less readily than it is for imipenem, as 2 mutations are required, i.e. a loss of OprD and overactivity of MexA-MexB-OprM. Moreover, the time needed for microorganisms to develop specific resistance mechanisms in the case of doripenem is shorter than for other carbapenems, resulting at present in the greater activity of this antibiotic in relation to selected pathogenic strains. It needs to be stressed that its activity was also observed in vitro in relation to *Streptococcus pneumoniae*, viridans streptococci, *Enterococcus faecalis* and methicillin-sensitive *Staphylococcus* spp. [26]. However, it needs to be stressed that in vitro activity does not automatically indicate the clinical efficacy of a drug and doripenem should be administered only to treat severe infections caused by notorious Gram-negative bacilli, to treat infections of mixed etiology or those caused by *P. aeruginosa* [26]. Another key stage within this study, apart from the analyses of activity of the clavulanic acid, was to determine MIC for all strains in the presence of the doripenem and clavulanic acid systems. In most investigated cases the application of the doripenem–clavulanic acid system enhanced the inhibitory effect towards microbial growth. In the case of clinical isolates the greatest reduction in MIC values from 4 μg/mL to 1 μg/mL was observed for *P. aeruginosa*, *P. vulgaris*

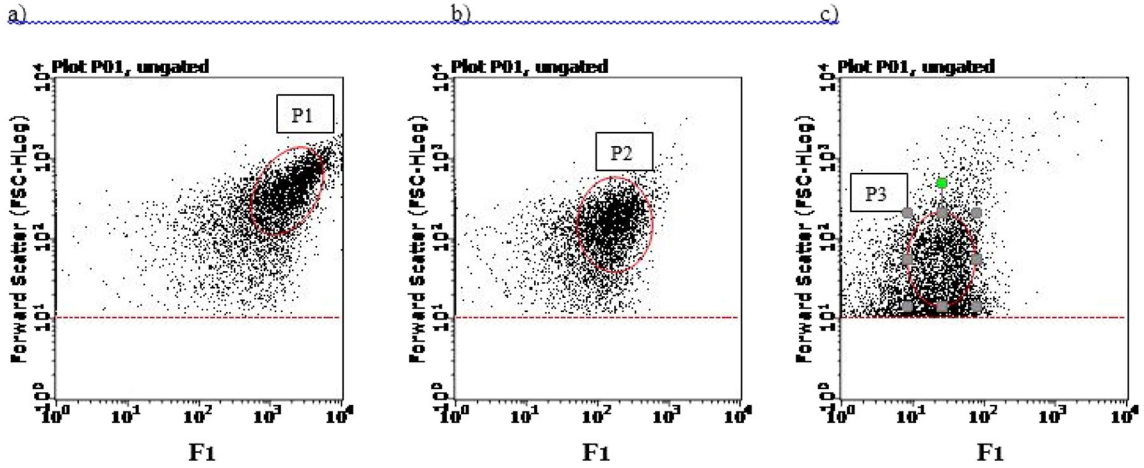


**Fig. 4.** Flow cytometry dot plots of F1 (PI, red fluorescence) of *P. aeruginosa* (ATCC 27853) treated with increasing concentrations of doripenem-clavulanic acid system; (a) 0.5 μg/mL; (b) 0.25 μg/mL; (c) 0.1 μg/mL. P1: dead cells; P2: cells damaged membranes; P3: live cells.

and *E. coli*. For species *A. baumannii*, no change was found in MIC values following the administration of the system in comparison to doripenem applied alone. Previous studies confirmed the activity of doripenem activity against a collection of 87 *A. baumannii* clinical [27]. The β-lactamase inhibitor - CA has also an intrinsic activity against *Acinetobacter* strains [8]. Therefore it is difficult to explain the lack of synergism for the activity of CA and doripenem. Nevertheless, the influence of different OXA-type carbapenemases cannot be excluded as a reason of these phenomena [28]. The absence of synergism in the case of CA and doripenem action against *E. faecalis* may be the effect of changes in pH of their solvent induced by the presence of CA [29]. Periodontal β-lactamase-negative pathogens such as *actinobacillus actinomycetemcomitans* and *E. faecalis* were more susceptible to co-amoxiclav than to amoxicillin alone.

Flow cytometry is a sensitive analytical technique which can rapidly monitor physiological states of bacteria (reproductively viable, metabolically active, intact, permeabilized) and which may be readily applied to the enumeration of viable bacteria in a biological sample [30]. In the case of Gram-negative bacteria already at lower concentrations of the complex we may see that a certain proportion of the population loses cell wall integrity. Only cells exhibiting the greatest resistance to the tested active substance remained viable. The difference in the effect of the doripenem-clavulanic acid system on Gram-negative and Gram-positive bacteria may also be connected with the difference in cell wall structure and thus with its permeability. Flow cytometry using respective fluorochrome tags is a useful tool presenting the metabolic status of the cell also on-line, while it also facilitates observations of selected mechanisms occurring in the cell under the influence of the active substance, which is impossible when using conventional culture methods. We need to stress here the increasingly extensive applicability of flow cytometry in clinical diagnostics, while current literature sources report comparisons between conventional methods assessing antimicrobial activity of active substance and flow cytometry. At present flow cytometry analysis provides an important supplementation and in the future it may become a competitive method to conventional techniques to determine antibiotic resistance. This is first of all thanks to the greatly reduced time of analysis and as a consequence rapid results, being crucial particularly in clinics [31] applied FCM to test antifungal susceptibility of *Aspergillus fumigatus* against three important fungicides (voriconazole, amphotericin B and itraconazole) used in therapy of aspergillosis. The results obtained within 3 to 4 h proved to be a reliable indicator of a drug's antifungal activity against *A. fumigatus* isolates and indicate a good correlation with the drug MICs obtained by the Clinical laboratory and Standard Institute broth microdilution method. Another example may be provided by studies on *M. tuberculosis*. Pyrizinamide susceptibility tested by FC was 93% concordant with the Bactec MGIT assay, and the former was conclusive within 24 h [32]. The rapid detection of the spectrum of microorganisms capable of synthesizing extended-spectrum beta-lactamases (ESBL) is a challenge for most clinical microbiological laboratories, as an inaccurate identification of ESBL producers leads to important clinical implications both in antibiotic therapy of infections and in the control. To meet these needs, a team of Faria-Ramos and co-workers [33] developed a flow cytometric assay. The proposed procedure makes it possible to identify strains capable of synthesizing extended-spectrum beta-lactamases (ESBL) as well as select an adequate inhibitor within a short time. Moreover, coupling the cytometer with a sorter makes it possible to separate cells exhibiting specific morphological and/or metabolic traits, which may facilitate selection of resistant strains and prove useful in further studies on these microorganisms.

It is also worth noting that a good therapeutic effect (eradication of the pathogen) can be achieved when between the consecutive doses of the drug, for about half the time, the concentration of antibiotic exceeds the MIC value. However, often during antibiotic therapy, the microorganisms for most of the time are exposed to a sub-threshold concentration, which means lower than the MIC value (sub-MIC; subminimal inhibitory concentration) [34]. It seems that it is very useful to know the influence of sub-threshold doses on microorganisms, especially in case of drugs which penetration to some tissues and organs is limited [35]. Although sub-threshold drug concentrations do not kill bacteria, they are able to induce changes in morphology and cell surface structures, inhibit toxins production and adhesion to host cells [34]. Therefore, the flow cytometry method may be unreliable in determining the MIC index. The fluorescence signal generated as the result of dye penetration into a morphologically changed cell may contribute to a false negative result. Therefore, the knowledge about the influence of sub-MICs doses of drugs on microorganisms in combination with pharmacodynamic indicators of the drug and the use of flow cytometry may turn out to be very useful in the rational antibiotic therapy [36,37].

## 5. Conclusions

Analysis of MIC levels supplies important information concerning microbial sensitivity to active substances, mainly in terms of limiting concentrations causing mortality or vitality of the tested species, which is essential when selecting appropriate antibiotic therapy. Moreover, supplementation of the conventional MIC method with flow cytometry provides insight into changes taking place on the cellular level following the application of the toxic agent, which may contribute to the broadening of our knowledge on microbial sensitivity to antibiotics.

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