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Original article

# Research of the activity of local anesthetics and antiseptics regarding clinical isolates of *Acinetobacter baumannii* as pathogens of postoperative infectious complications

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**Abstract.** *Background:* About 60% of all nosocomial infections are caused by microorganisms found in biofilms. *Acinetobacter baumannii,* as a pathogen of nosocomial infections, occur more frequently in surgical hospitals. The aim of the study was to study the in vitro formation of A. baumannii monotype biofilms under the influence of local anesthetics and antiseptics. *Materials and methods:* The antimicrobial activity of local anesthetics (0.25-0.5%, bupivacaine, 2.0% lidocaine, 0.75% ropivacaine) and antiseptics (decamethoxine 0.1%, octenidine 0.1%, chlorhexidine 0.05%) against clinical strains of *A. baumannii* and studied their ability to produce biofilms. *Results:* The bacteriostatic effect of local anesthetics on *A. baumannii* within the current concentrations of these drugs, which provide analgesic effect, has been proven. The given results indicate that *A.baumanni* cultures have a pronounced ability to form biofilms. Clinical strains of opportunistic microorganisms *A. baumannii* circulating in the hospital environment are highly sensitive to antiseptics decamethoxine and octenidine, chlorhexidine has a weak bactericidal effect against acinetobacteria. The results of studying the effect of local anesthetics on the process of formation of the strain "young" biofilms showed the presence of a dose-dependent effect. The greatest inhibitory activity against "young" biofilms was detected under the combined action of the antiseptic decamethoxine (in concentrations, not exceeding 3.9 μg / ml) and anesthetics (OD-0,199-0,223) ($p < 0,05$). *Conclusions:* Scientific research on various aspects of the formation (or destruction) of bacterial biofilms is a relevant and promising area that will change approaches to the prophylaxis and treatment of a number of infections, including postoperative infectious complications. (www.actabiomedica.it)

**Key words:** *Acinetobacter baumannii*, local anesthetics, antiseptics, biofilms.

## Introduction

Infectious diseases associated with providing medical care (*a hospital-acquired infection*), also known as nosocomial infections (NI) are relevant today to hospitals of various profiles. Thus, according to the World Health Organization (WHO), NIs cause 37,000 deaths in Europe yearly (1). On average, from 7 to 10% of patients get at least one of these infections during their stay in medical institutions (1). The vast majority of nosocomial infections are developed as a result of infection of patients with nosocomial strains. Even though

the etiological profile of such infections differs depending on the specifics of the hospital or its departments, most often such strains are isolated from patients in the intensive care unit and in the early postoperative period. Thus, up to 90% of all NI are of bacterial origin (2).

In the last decade, gram-negative flora is dominated in the etiological structure of postoperative purulent-septic complications. The most common among them are enterobacteria and non-fermenting gram-negative bacteria (11). A distinctive feature of these bacteria, especially nosocomial strains, is high resistance to antibiotics. As a pathogen of nosocomial infections, *Acinetobacter baumannii* is increasingly found in surgical hospitals of Ukraine (4). These pathogens are also common in the United States, Asia and Europe (5-6). The clinical significance of the genus *Acinetobacter* in the etiology of nosocomial pneumonia is important that is associated with pulmonary ventilation. The presence of these pathogens among clinical isolates detected from wounds is noticed. They are mostly found in the chronic course of purulent-inflammatory process, as well as in nosocomial infection or colonization on the background of long-term antimicrobial therapy (4-6). At present, the etiological role of *A. baumannii* has been proven in causing infections such as pneumonia, endocarditis, skin and soft tissue infections, peritonitis, bloodstream infections, etc.. (5-6).

Bacteria of the genus *Acinetobacter* are low-virulent. Microorganisms of this species are found everywhere, including the skin of medical workers, medical equipment, increasing the risk of contamination of patients' wounds in the postoperative period. Significant species diversity of bacteria of the genus *Acinetobacter* is known; the ability (all known species) to cause infectious processes in humans is proven, but *A. baumannii* accounts for about 80% of infections. The biggest problem in the treatment of these nosologies so far is the spread of antibiotic resistance among *A. baumannii* isolates. Moreover, the development of both carbapenem and multidrug-resistant strains is observed (7).

According to foreign and domestic researchers, the formation of biofilms by acinetobacteria plays an important role in the development of antibiotic resistance. The formed extracellular polysaccharide matrix complicates the penetration of antimicrobial drugs into the internal loci of biofilms and leads to the fact that acinetobacteria in the deep layers of biofilms become inaccessible to therapeutic concentrations of antibiotics (8).

Increased resistance to antibiotics in hospitals leads to ineffective antimicrobial therapy, increasing the duration of hospitalization and treatment costs, increasing mortality from infections. At the same time, there is a global decline in the development, research and registration of new antimicrobial drugs. All this dictates to the medical community the need to take urgent measures to control the level of antibiotic resistance and preventive measures, using alternative antimicrobials.

The aim is to study the antimicrobial effect of local anesthetics and antiseptics on clinical isolates of *A. baumannii* as pathogens of postoperative infectious complications, and their ability to form monotype biofilms.

## Materials and methods

The research was conducted on the basis of the bacteriological laboratory of the Department of Microbiology of National Pirogov Memorial Medical University, Vinnytsia from April to November 2020.

A total number of patients, included in the given study – 68, all with postoperative infectious complications, regardless of age, sex, and length of hospital stay. All patients enrolled in the study underwent biomaterial collection from the wound surface during tampon dressing prior to local antimicrobial use. The tampons were immersed in Stewart's transport medium. Then in the bacteriological laboratory tampons were inoculated into 10 ml of saline, prepared a series of dilutions followed by seeding on nutrient agar medium and incubated at 37° C for 24 hours. Among the studied isolates as a result of final identification by morphological, tinctorial, cultural and biochemical characteristics of acinetobacteria, 41 strains were identified as *Acinetobacter baumannii.*

It was studied the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of local anesthetics (bupivacaine 0.5%, lidocaine 2.0%, ropivacaine 0.75%) and antiseptics (decamethoxine 0.1%, octenidine 0.1%, chlorhexidine 0.05%) on clinical isolates of *A.baumannii* (n=41) detected from patients with postoperative

infectious complications by the method of double serial dilutions.

In the clinical isolates *A.baumannii* it was studied the ability to produce biofilms in the presence of subbacteriostatic concentrations of anesthetics and antiseptics, which were 2-4 times smaller than the MIC, using the spectrophotometric method MtP-test by G.D. Christensen. Polystyrene microtiter 96-well plates were used to evaluate the efficiency of A. baumannii film formation on a solid surface. The wells of the plates were introduced liquid medium inoculated with the test microorganism in a volume of 150 μl at a dilution of 1:10, then the plate was incubated at 37° C for 2 and 3 days. After incubation, the medium was removed from the wells together with the planktonic cells of the microorganism and washed once with saline. The biofilm, grown in the wells, was stained with 100 μl of a 0.1% aqueous solution of gentian violet for 45 min at room temperature. 200 μl of 96% ethyl alcohol was added to the film in the wells of the plate and left at room temperature for 45 min to extract the dye with alcohol. Biofilm formation was studied by spectrophotometric analysis (G. Toole, R. Kolter, 1998) on a STAT FAX®4300 spectrophotometer (Netherlands) at a wavelength of 620 nm. The results were processed using a special computer program. Quantitative expression of the degree of formation of biofilms was the values of optical density (OD), which were measured on a spectrophotometer. The significance of differences in the obtained indicators was determined by Student's test (9,10).

## Results

Clinical isolates of *A. baumannii* were found to be sensitive to all three anesthetics studied. The bacteriostatic effect of bupivacaine, lidocaine and ropivacaine on *A. baumannii* within the current concentrations of these drugs, which provide analgesic effect, has been proven. The advantages of bacteriostatic properties of 0.5% bupivacaine over the studied pathogens were established, the drug had an antimicrobial effect at half the dose of 3900 μg / ml (2500 - 5000 μg / ml) ($p < 0.001$) and showed a bactericidal effect against the clinical isolate *A.baumanni* (MBCs 2500-3750 μg / ml). Clinical isolates of acinetobacteria showed sensitivity to high concentrations of lidocaine, which were within the concentrations of the active substance in the finished dosage form (MIC 5000-1000 μg / ml). It was found that lidocaine had a bactericidal effect only on some clinical isolates of *A. baumannii* in the presence of MBCs > 10000 μg / ml. Ropivacaine has the lowest antimicrobial properties for the studied pathogens. Thus, ropivacaine showed only a weak inhibitory effect on *A. baumannii* at high concentrations of the drug (MIC 7500 μg / ml) and did not have a bactericidal effect (Fig. 1).

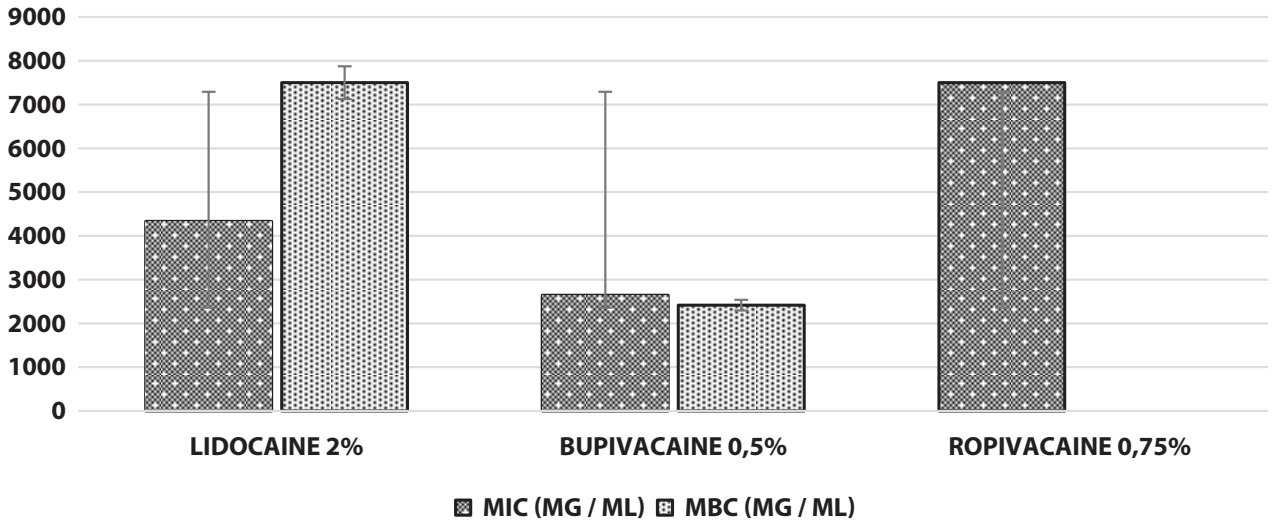


**Figure 1.** Characteristics of bacteriostatic (MIC) and bactericidal (MBC) action of anesthetics on strains of opportunistic pathogens A. baumannii, μg / ml.

The study of antiseptics showed significant advantages of bacteriostatic and bactericidal properties of decamethoxine and octenidine in comparison with chlorhexidine over clinical strains of *A. baumannii* ($p < 0.05$). The obtained data showed that the MBC of decamethoxine and octenidine relative to *A. baumannii* were 1.7 and 2.3 times lower than that of chlorhexidine, respectively ($p < 0.001$) (Fig. 2).

Octenidine was found to have a bacteriostatic effect on *A. baumannii* at MIC of 4.45 ± 2.20 μg / ml, and bactericidal properties at concentrations of 15.79 ± 7.8 μg / ml. Decamethoxine also had high bacteriostatic and bactericidal properties against clinical isolates of *A. baumannii* (MIC 10.45 ± 3.8 μg / ml and MBC 19.5 ± 8.9 μg / ml, respectively), but antimicrobial properties compared with octenidine were 2.3 times lower ($p < 0.001$) and 1.24 times lower, respectively ($p < 0.001$). Chlorhexidine showed bactericidal properties in the presence of high concentrations, which reached 33.73 ± 15.92 μg / ml and bacteriostatic properties at concentrations of 27.87 ± 7.92 (Fig. 2).

In addition, the antimicrobial properties (MIC and MBC) of anesthetics in the presence of antiseptic concentrations were analyzed, that were 4 times lower than the MIC (decamethoxine-3.9 μg / ml; octenidine -1.95 μg / ml and chlorhexidine -1.95 μg / ml) (Fig. 3).

It was found that in the presence of decamethoxine, the bacteriostatic and bactericidal action of the anesthetic lidocaine did not differ from the previous values without the addition of antiseptics, and averaged MIC 6800 μg / ml (5000-1000 μg / ml) and MBC 10000 μg / ml. Bupivacaine showed the best antimicrobial properties when decamethoxine was added, so the MIC averaged 3000 ±250 μg / ml, and the high bactericidal effect of bupivacaine was determined at MBC 2500 ± κ μg / ml. Given that ropivacaine did not have a bactericidal effect and had a weak bacteriostatic effect, studies with decamethoxine were not performed (Fig. 3).

Octenidine enhanced the antimicrobial effect of the studied anesthetics. Thus, when a subbacteriostatic dose of octenidine was added to lidocaine, the MIC of lidocaine averaged 2,500 μg / ml, for bupivacaine the MIC was 632 μg / ml, and ropivacaine enhanced the bacteriostatic effect, and was 938 μg / ml.

Despite the lowest antimicrobial effect of chlorhexidine compared to decamethoxine and octenidine, when its subbacteriostatic concentration was added to anesthetics, it had a potentiating antimicrobial effect. Thus, when adding a subbacteriostatic concentration of chlorhexidine to bupivacaine, the MIC of the latter was 1250 μg / ml, for ropivacaine - MIC 3750 μg / ml, lidocaine had the weakest bacteriostatic effect and the MIC averaged 10,000 ±500 μg / ml.

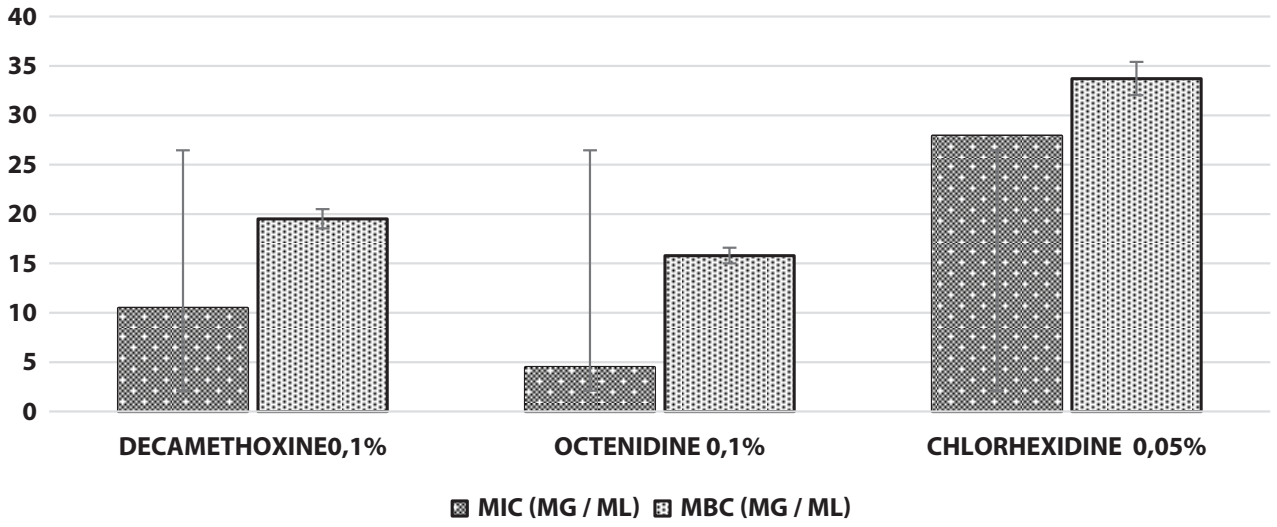


**Figure 2.** Characteristics of bacteriostatic (MIC) and bactericidal (MBC) action of antiseptics on strains of opportunistic pathogens A. baumannii, μg / ml.

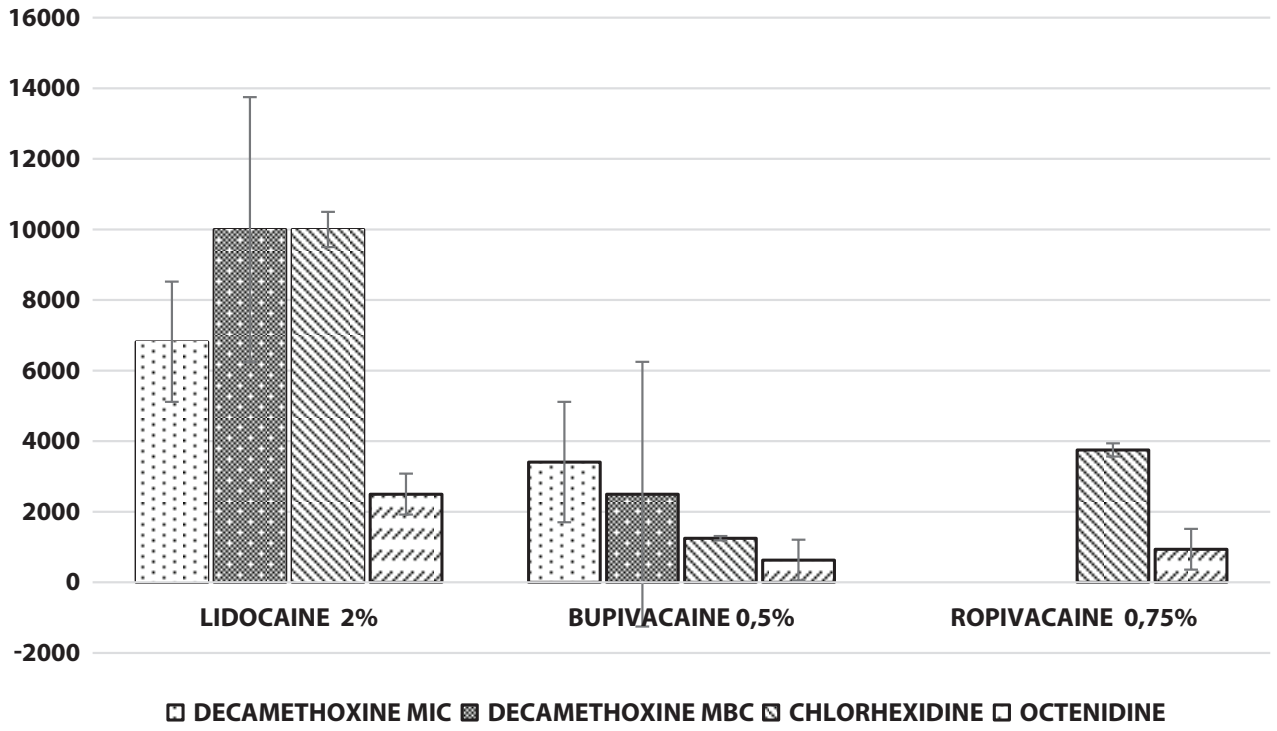


**Figure 3.** Characteristics of bacteriostatic properties (MIC) of local anesthetics in the presence of subbacteriostatic doses of antiseptics against *A. baumannii*, μg / ml.

To study the formation of biofilms on the surface of the wells under the action of anesthetics and antiseptic decamethoxine, staining with gentian violet was performed and the optical density (OD) of biofilms of each isolate of *A. baumannii* was studied (Table 2,3). Relative to the control well, the optical densities of the biofilms were divided into dense, moderate density and low density. The results were evaluated after 24 hours and 48 hours (Fig.4 A, B).

In general, after 24 hours of study, the bactericidal properties of these isolates showed all antiseptics, both alone and in the presence of subbacteriostatic concentrations of decamethoxine, not exceeding 3.9 **μg** / ml. The bacteriostatic effect of this antiseptic was separately determined in the presence of 0.8 ± 0.01 **μg** / ml. The relative rate of film formation of *A. baumannii* (1,204 OD) was determined. It was found that in the presence of 1.0% lidocaine and 0.5% lidocaine, the ability to form biofilms was inhibited 1,151 and 1,117 times, respectively. In the presence of decamethoxine with 1% and 0.5% lidocaine, the relative rate of film formation was 1.036 BS and 1.041 OD, which indicated a decrease in *A.baumannii* film production by 1.162 and 1.156 times (Fig. 5).

In bupivacaine 0.125%, the relative rate of film formation after 24 hours was 1.138 OD, with the addition of decamethoxine, it decreased to 1.097 OD.

It was found that in the presence of 0.063% bupivacaine, the biofilm was dense, the relative value was 1,189 OD, in the presence of decamethoxine the biofilm was characterized by low density and the relative value was 1,143. (Fig. 6).

After 48 hours, the relative index/rate of biofilm formation of *A. baumannii* increased to 1,278 OD and the optical density of the biofilm averaged 0.232 ± 0.006 OD. In the presence of local anesthetics and with the addition of a subbacteriostatic dose of the antiseptic decamethoxine, the optical densities of biofilms were low (Table 2).

The highest ability to inhibit the biofilm formation of *A. baumannii* was found by adding 1% and 0.5% to the lidocaine wells, where the relative value was 1.056 OD and 1.071 OD, respectively. When decamethoxine was added to lidocaine 1% and lidocaine 0.5%, the relative values were 1,040 OD and 1,054 OD. (Fig. 5)

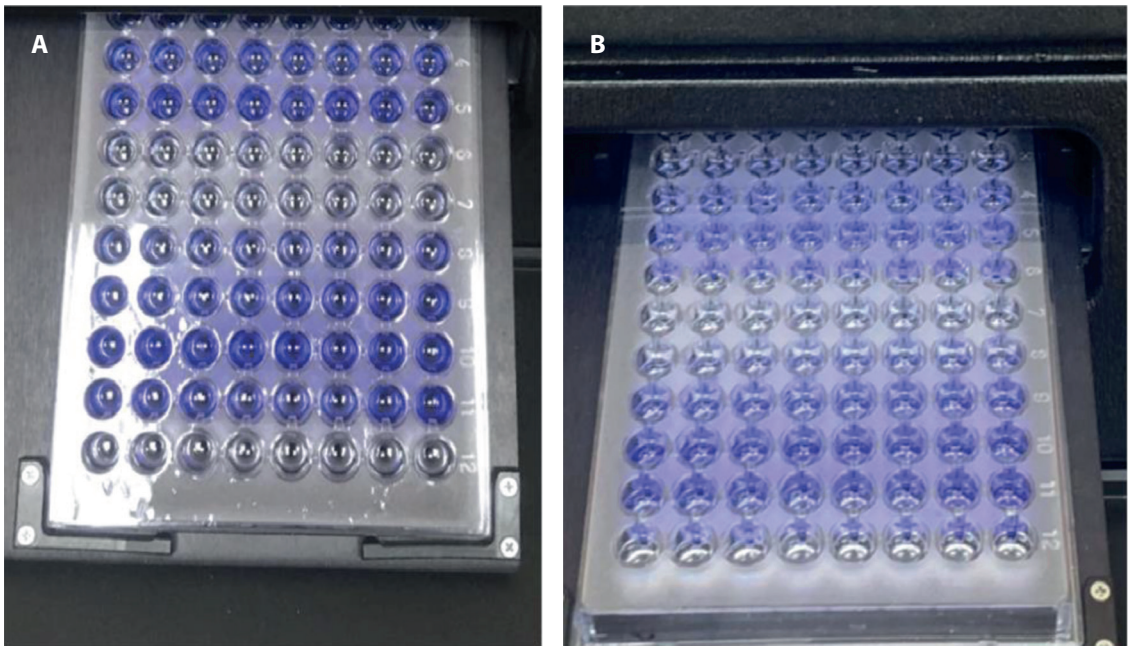


**Figure 4.** Microtiter plate showing different grades of biofilm formation:
A) after 24 hours; B) after 48 hours.

**Table 1.** Characteristics of the effect of local anesthetics and antiseptic decamethoxine on the biofilm formation of clinical isolates of A. baumannii after 24 hours, OD

| ***A. baumannii*** **(n=41)** | **Culture Control** | **L1%** | **L 0,5%** | **Bup. 0,125%** | **Bup. 0,063%** | **L1%+ DKM** | **L0,5% +DKM** | **Bup 0,125% +DKM** | **Bup 0,063% +DKM** | **DKM sub** |
|---|---|---|---|---|---|---|---|---|---|---|
| M±m* | 0,245± 0,006 | 0,207± 0,001 | 0,214± 0,005 | 0,225± 0,001 | 0,238± 0,004 | 0,205± 0,001 | 0,206± 0,001 | 0,221± 0,001 | 0,228± 0,001 | 0,232± 0,004 |
| min-max** | 0,240-0,250 | 0,204-0,220 | 0,204-0,22 | 0,219-0,228 | 0,230-0,243) | 0,199-0,213 | 0,204-0,210 | (0,218-0,23) | 0,221-0,238) | 0,222-0,241 |
| characteristics of biofilms | control | Low density | Low density | Low density | dense | Low density | Low density | Low density | Low density | dense |

Notes: * - mean values and standard deviation of the mean; ** - range of optical density values from the smallest to the largest.

It was found that the average film formation of A. baumannii (1,278 OD) was almost unchanged in the presence of 0.063% bupivacaine (1,227 OD) and 0.125% bupivacaine (1,192 OD). In the presence of a subbacteriostatic dose of decamethoxine, the rate/index of biofilm formation also remained constant (Fig. 6).

It is proved that the simultaneous use of the antiseptic decamethoxine (1/4 of the minimum bacteriostatic concentration) with anesthetics, there is an increase in the inhibitory effect of the latter on the biofilm formation of *A. baumannii* in the first 24 hours, after 48 hours the bacteriostatic effect of decamethoxine decreased.

## Discussion of results

The formation of biofilms by microorganisms is one of the most important mechanisms of survival in an aggressive environment and, as a consequence, the chronicity of the process and the development of antibiotic resistance. Therefore, the development of new approaches to combating biofilm formation is an important area of medicine and microbiology. For all their diversity, such approaches require at least two conditions: a comprehensive approach to effective action on the microflora and safe effects on the human body (11).

**Table 2.** Characteristics of the effect of local anesthetics and antiseptic decamethoxine on the biofilm formation of clinical strains of A. baumannii after 48 hours, OD

| ***A.baumannii*** **(n=41)** | **Culture Control** | **L1%** | **L 0,5%** | **Bup. 0,125%** | **Bup. 0,063%** | **L1%+ DKM** | **L0,5% +DKM** | **Bup 0,125% +DKM** | **Bup 0,063% +DKM** | **DKM sub** |
|---|---|---|---|---|---|---|---|---|---|---|
| M±m* | 0,232± 0,006 | 0,206± 0,001 | 0,209± 0,005 | 0,234± 0,001 | 0,244± 0,004 | 0,200± 0,001 | 0,218± 0,001 | 0,230± 0,001 | 0,248± 0,001 | 0,240± 0,004 |
| min-max** | 0,214-0,258 | 0,199-0,214 | 0,2-0,214 | 0,225-0,238 | 0,240-0,248) | 0,199-0,213 | 0,207-0,224 | (0,221-0,30) | 0,237-0,257) | 0,222-0,258 |
| characteristics of biofilms | control | Low density | Low density | dense | dense | Low density | Low density | dense | dense | dense |

*Notes: * – mean values and standard deviation of the mean; ** – range of optical density values from the smallest to the largest.*

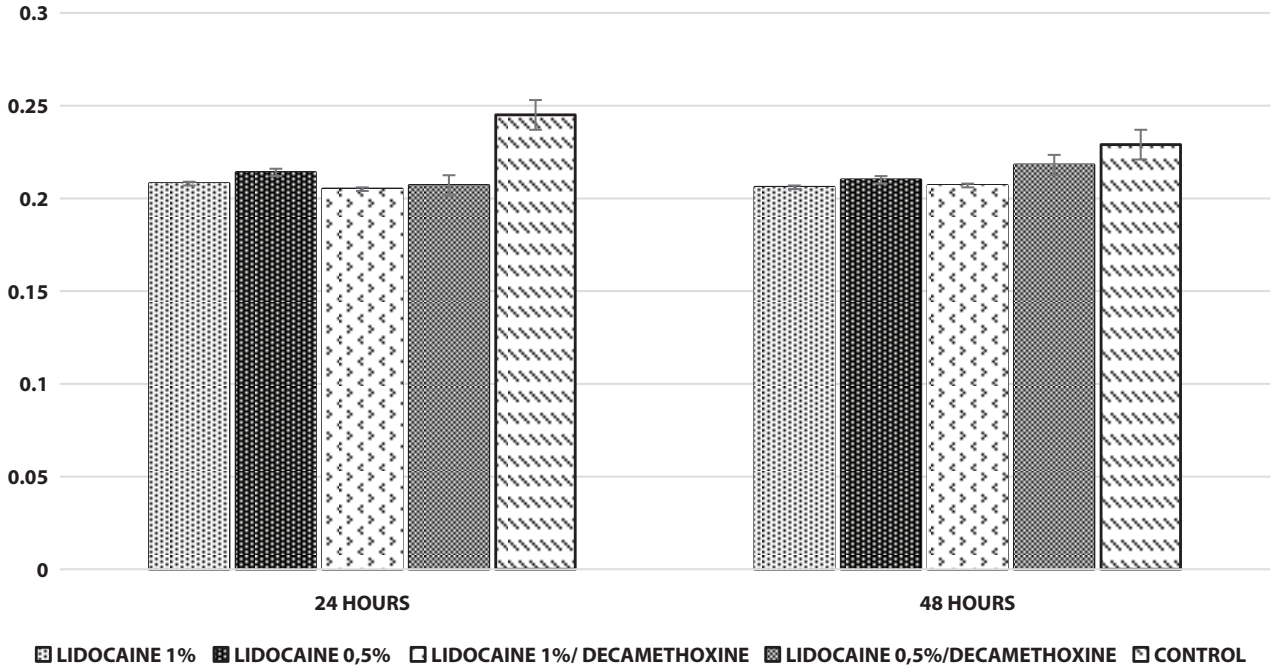


**Figure 5.** Alteration capacity for biofilm formation of clinical isolates of A. baumannii in the presence of 1.0% lidocaine and 0.5% bupivacaine and with the addition of subbacteriostatic concentrations of the antiseptic decamethoxine (OD); Control - control of culture.

In our studies, the sensitivity of planktonic forms of *A. baumannii* to the antiseptic chlorhexidine, QAC antiseptics decamethoxine, octenidine, and local anesthetics. We have found that *A. baumannii* is sensitive to anesthetics and antiseptics mostly in clinical concentrations. The pronounced bactericidal effect of decamethoxine, octenidine and 0.5% bupavacaine and moderate bacteriostatic properties of 0.25% bupivacaine, 0.75% ropivacaine, 1% lidocaine were established. When adding subbacteriostatic concentrations of antiseptics to 0.5% bupivacaine, an increase in its bactericidal effect was observed.

The weakest bacteriostatic properties were determined in ropivacaine, with no bactericidal properties in the drug. Despite the weakest antimicrobial effect on A. baumannii chlorhexidine, in contrast to decamethoxine and octenidine, when adding its subbacteriostatic concentration to anesthetics, there is an increase in the bacteriostatic effect of the latter.

Cultures of A. *baumannii* that were isolated from infected wounds of postoperative patients were characterized by a similar ability to film formation ($p<0,005$). These studies coincide with the literature on the ability to form biofilms by hospital strains.

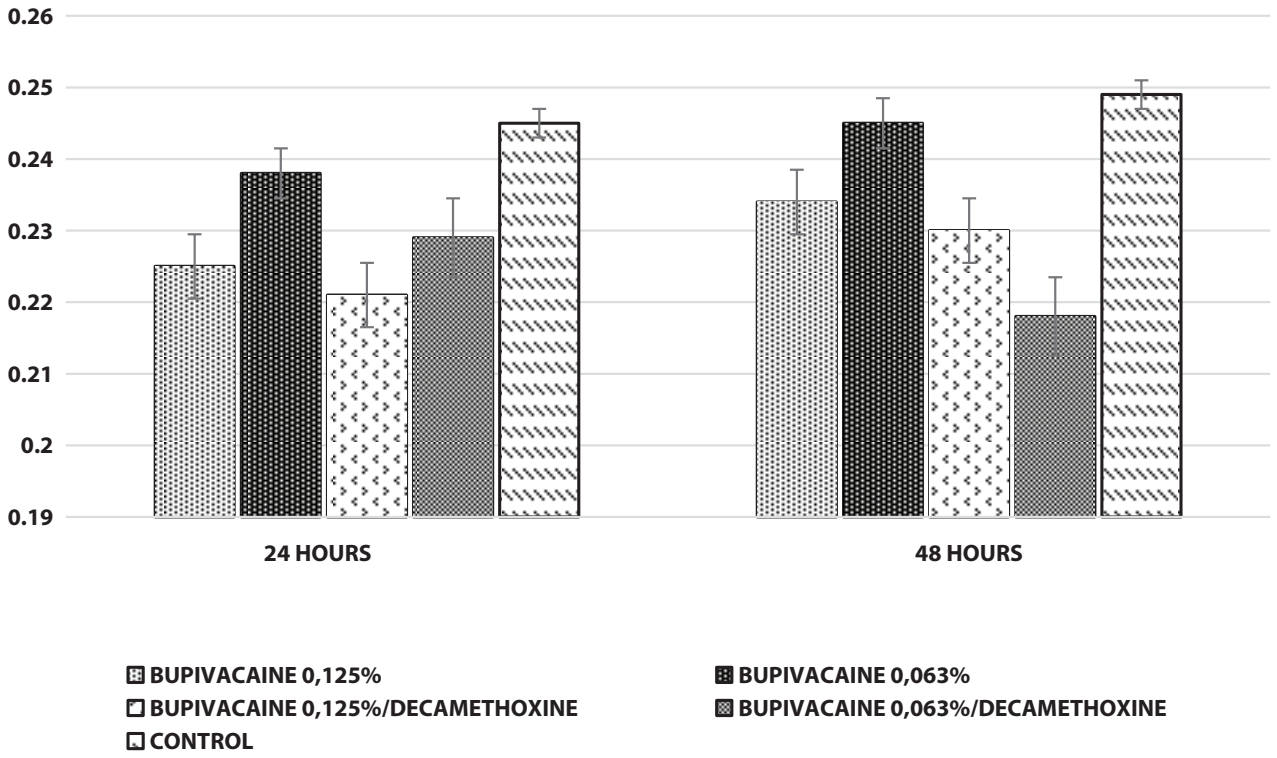


**Figure 6.** Alteration capacity for biofilm formation of clinical isolates of A. baumannii in the presence of 0.125% bupivacaine and 0.063% bupivacaine and with the addition of a subbacteriostatic concentration of the antiseptic decamethoxine (OD); Control - control of culture.

It is shown that the most significant ability to form film in strains isolated from bronchoalveolar lavage, vascular catheters and wounds (12). In the study of both planktonic and biofilm forms of A. *baumannii* to lidocaine 2%, 1% and 0.5%, a pronounced antimicrobial effect and low density of the biofilm (0.207 ± 0.010) were observed both after 24 hours and after 48 hours.). When decamethoxine was added to lidocaine in ¼ MIC, the antimicrobial effect was enhanced. In a study of the efficacy of local anesthetics bupivacaine 0.125% and 0.063% for both planktonic and film forms of A. *baumanni*: anesthetics in clinical concentrations showed antimicrobial effect and had a suppressive effect on the acinetobacter capacity to produce biofilm, as it was evidenced from low bioavailability and with the addition of ¼ MIC decamethoxine, however, compared with lidocaine, biofilm production in the presence of bupivacaine was higher.

## Conclusions

The given results indicate that *A.baumanni* cultures have a pronounced ability to form biofilms. Clinical strains of opportunistic microorganisms *A. baumannii* circulating in the hospital environment are highly sensitive to antiseptics decamethoxine and octenidine, chlorhexidine has a weak bactericidal effect against acinetobacteria. There is inhibition of their growth and reproduction in the presence of clinical concentrations of modern local anesthetics (bupivacaine 0.5%, lidocaine 2.0%), which are used in modern medical practice.

The drug for local anesthesia ropivacaine provides a weak bacteriostatic effect only on the planktonic form of acetobacteria, one of the manifestations of the stability of clinical isolates which is the active production of dense biofilm in the presence of this local amide-type anesthetic.

While applying simultaneously QAC antiseptic decamethoxine (1/4 of the minimum bacteriostatic concentration) with anesthetics, there is an increase in the inhibition of recent biofilm formation in *A. baumannii*.

The results indicate that in the fight against postoperative wound infection caused by clinical isolates of *A. baumannii*, which produce biofilms as resistance factors, it is advisable to use in the area of the entrance/ input gate local anesthetics (libocaine, bupivacaine) in combination with QAC antiseptics as a universal approach to effective prevention of postoperative wound infectious and inflammatory complications.

**Conflict of Interest:** Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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