COMPARATIVE STUDY OF ANTIMICROBIAL PROPERTIES OF BIOMATERIALS AND DRESSINGS BASED ON ANTISEPTICS AGAINST GRAM-NEGATIVE BACTERIA AS PATHOGENS OF WOUND INFECTIONS

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The antimicrobial stewardship strategy stems from the need to counter resistant microorganisms and requires judicious use of available antimicrobial agents and treatment approaches to prevent the spread of resistance [9]. On the other hand, topical antibiotics are considered inappropriate or ineffective in combating wound biofilm [10]. Since the effective treatment of wounds today depends on non-antibiotic antimicrobial agents, scientists hope to use antiseptics in the management of patients with wound infections, which can replace, supplement (in the case of deep wound infections) or enhance (potentiate) the effect of antibiotics to prevent the spread of antibiotic-resistant strains [11-16].

But not only the activity of the medicinal compound, but also the method of delivery affects numerous factors that contribute to therapeutic effectiveness [17]. Therefore, active wound dressings based on biomaterials made of biocompatible polymers with the addition of effective antimicrobial compounds deserve special attention [18].

Such a polymer as alginate (Alg), due to favourable properties such as biocompatibility and ease of gelation, has become particularly attractive for the development of biomaterials [19]. Polyvinyl alcohol (PVA), in the manufacture of composite biomaterials, shows synergism with additional functional components, including Alg, in improving wound healing and improving the physicochemical properties of biomaterials [2, 20].

Different forms of biomaterials (films, hydrogels, foams, etc.) with integrated antimicrobial drugs were
developed to enhance the antibacterial effect and to ensure the controlled release of the active compound [3]. The development and implementation of biomaterials in the form of bioactive or therapeutic wound dressings with integrated bioactive molecules (antiseptics) for achieving controlled antibacterial treatment is relevant and promising.

The aim of the study.

To investigate the antimicrobial activity of new biomaterials developed on the basis of decamethoxine, polyvinyl alcohol and calcium alginate, and modern commercially available antimicrobial wound dressings against reference and clinical strains of causative agents of healthcare-associated wound infections, K. pneumoniae, A. baumannii and P. aeruginosa.

Object and research methods.

For the study, developed biomaterials with decamethoxine and commercially available wound dressings containing antiseptics were used, which were marked with the appropriate numbers: №1 Decametoxine (DCM), №2 Decametoxine (DCM), №3 Decametoxine (DCM), №4 – Suprasorb® X + PHMB, №5 – SILVERCEL® Hydro-Alginate, №6 – Urgotul SSD®, №7- GUANPOLI-SEPT®, №8 – Bétadine® TULLE 10% DRESSING. The initial diameter of all tested materials was 6 mm.

Samples of biomaterials with decamethoxine were made from calcium alginate (Ca-Alg), polyvinyl alcohol (PVA) and antiseptic (0.05% DCM) by solvent casting method [21]. The composition of the studied samples: №1 Decametoxine (DCM), №2 Decametoxine (DCM), №3 Decametoxine (DCM), №4 – Suprasorb® X + PHMB, №5 – SILVERCEL® Hydro-Alginate, №6 – Urgotul SSD®, №7- GUANPOLI-SEPT®, №8 – Bétadine® TULLE 10% DRESSING. The initial diameter of all tested materials was 6 mm.

Research results and their discussion.

As a result of the study of the activity of biomaterials against reference and clinical strains of K. pneumoniae, A. baumannii and P. aeruginosa, high antimicrobial properties of materials based on decamethoxine and commercially available wound dressings were revealed (tables 1, 2).

The reference strain K. pneumoniae ATCC 700603 was most susceptible to Suprasorb® (№4), Guanpolisept® (№7), Bétadine® (№8) and samples with DCM №1-3. Among them, the action of samples with decamethoxine №1 and №2 was determined to be the most effective (table 1). ZOIs around DCM №1 and DCM №2 exceeded those around silver-containing materials (№5 and №6) by 2.34-2.84 times (р<0.001). Dressings based on polyhexanide Suprasorb® and Guanpolisept® were more effective than silver-containing dressings by 2.24-2.68 (р<0.001) and 1.84-2.21 times (р<0.001), respectively. Susceptibility of K. pneumoniae ATCC 700603 to biomaterial with povidone-iodine was higher, compared to susceptibility to silver-containing ones by 1.48-1.84 (р<0.001) and 1.84-2.21 times (р<0.001), respectively. Susceptibility of K. pneumoniae ATCC 700603 to biomaterial with povidone-iodine was higher, compared to susceptibility to silver-containing ones by 1.48-1.84 (р<0.001) and 1.84-2.21 times (р<0.001), respectively.

Thus, based on the average ZOIs of K. pneumoniae ATCC 700603, the following activity of biomaterials was established (from the most effective):

1. №1 DCM > №3 DCM > №2 DCM > №4-Suprasorb® > №7-Guanpolisept® > №8-Bétadine® >№6-Urgotul® > №5-Silvercel®.

Clinical strains turned out to be much more tolerant, but the tendency of K. pneumoniae to show sensitivity to certain biomaterials was also observed in clinical strains. Antimicrobial properties of Suprasorb® and samples with decamethoxine were most actively demonstrated (table 2, fig.). Biomaterial №4 – Suprasorb® was determined to be the most active biomaterial based on ZOI data, but its average ZOI values did not differ significantly from those of biomaterials based on decamethoxine №1, №2, and №3 (р>0.05), so their effectiveness should be considered equivalent.

The ranking of the effectiveness of biomaterials against clinical strains of K. pneumoniae based on the average values of ZOIs (from the most effective) was as follows (table 2):

1. №4 – Suprasorb® > №1 DCM > №3 DCM > №2 DCM > №7-Guanpolisept® > №8 – Bétadine® > №6-Urgotul® > №5 – Silvercel®

Table 1 – Effectiveness of antimicrobial biomaterials against reference strains of K. pneumoniae, A. baumannii and P. aeruginosa (mean values of ZOIs, M±m, mm)

<table>
<thead>
<tr>
<th>Biomaterials</th>
<th>Strains</th>
<th>K. pneumoniae AC 700603</th>
<th>A. baumannii BAA-747</th>
<th>P. aeruginosa AC 27853</th>
</tr>
</thead>
<tbody>
<tr>
<td>№1 DCM</td>
<td>20.18±0.22</td>
<td>15.61±0.01</td>
<td>13.47±0.28</td>
<td></td>
</tr>
<tr>
<td>№2 DCM</td>
<td>19.13±0.19</td>
<td>15.41±0.01</td>
<td>14.01±0.36</td>
<td></td>
</tr>
<tr>
<td>№3 DCM</td>
<td>19.98±0.1</td>
<td>15.76±0.02</td>
<td>13.78±0.19</td>
<td></td>
</tr>
<tr>
<td>№4 Suprasorb®</td>
<td>19.06±0.19</td>
<td>16.31±0.15</td>
<td>11.02±0.18</td>
<td></td>
</tr>
<tr>
<td>№5 Silvercel®</td>
<td>7.1±0.02</td>
<td>7.61±0.03</td>
<td>7.2±0.25</td>
<td></td>
</tr>
<tr>
<td>№6 Urgotul®</td>
<td>8.51±0.04</td>
<td>8.43±0.04</td>
<td>7.82±0.29</td>
<td></td>
</tr>
<tr>
<td>№7 Guanpolisept®</td>
<td>15.69±0.01</td>
<td>14.11±0.21</td>
<td>7.14±0.32</td>
<td></td>
</tr>
<tr>
<td>№8 Bétadine®</td>
<td>12.56±0.26</td>
<td>9.08±0.13</td>
<td>10.13±0.004</td>
<td></td>
</tr>
</tbody>
</table>

(p). The Susceptibility of each strain was studied in four replicates.
As a result of the study of the activity of biomaterials against reference and clinical strains of \textit{A. baumannii}, similar patterns were revealed: biomaterials based on decamethoxime and polyhexanide (Suprasorb®, Guanpolisept® and No. 1-3 DCM) were the most effective, but the difference in the susceptibility of reference strains compared to clinical ones was not as pronounced as for \textit{K. pneumoniae} (\textit{tables 1, 2}).

The highest efficiency against the reference strain \textit{A. baumannii BAA-747} was observed in Suprasorb® wound dressing and the difference in values was significant (from \textit{p<0.01} to \textit{p<0.001}). ZOIs against clinical strains of \textit{A. baumannii} were also found to be the greatest for Suprasorb®, but the mean ZOIs were not significantly different from those of decamethoxime-based biomaterials №1, №2, and №3 (\textit{p>0.05}), and thus they were equally effective (\textit{table 2, fig.}).

The multiplicity of the difference in ZOI values of clinical strains for Suprasorb® and silver-containing materials was 1.93-2.21 times (\textit{p<0.01}), and in comparison with povidone-iodine dressing – 1.72 times (\textit{p<0.01}). ZOIs around decamethoxime-containing samples were 1.81-2.16 times larger compared to silver-containing ones (\textit{p<0.001}) and 1.62-1.68 times larger compared to povidone-iodine dressings (\textit{p<0.001}).

Ranking scale of the effectiveness of biomaterials in relation to the reference strain \textit{A. baumannii BAA-747} (from the most active):

\begin{itemize}
  \item \textbf{№ 4} – Suprasorb®
  \item \textbf{№ 3} DCM > \textbf{№ 1} DCM > \textbf{№ 2} DCM
  \item >\textbf{№ 7} Guanpolisept® > \textbf{№ 8} – Bétadine® > \textbf{№ 6} Urgotul® > \textbf{№ 5} Silvercel®
  \item With regard to clinical strains –
  \item \textbf{№ 4} – Suprasorb® > \textbf{№ 2} DCM > \textbf{№ 3} DCM > \textbf{№ 1} DCM >\textbf{№ 7} Guanpolisept® > \textbf{№ 8} – Bétadine® > \textbf{№ 6} Urgotul® > \textbf{№ 5} – Silvercel®
\end{itemize}

The smallest ZOIs were observed for \textit{P. aeruginosa} (\textit{Tables 1, 2}). \textit{Pseudomonas aeruginosa} is the most antimicrobial-resistant wound pathogen. Reference strains have natural, and clinical strains have natural and acquired resistance to biocides [25-27].

Biomaterials according to their effectiveness against the reference strain \textit{P. aeruginosa ATCC 27853} (based on average ZOIs) were distributed as follows (from the most effective):

\begin{itemize}
  \item \textbf{№ 2} DCM > \textbf{№ 3} DCM > \textbf{№ 1} DCM > \textbf{№ 4} Suprasorb®
  \item >\textbf{№ 8} – Bétadine®
  \item \textbf{№ 6} Urgotul® > \textbf{№ 5} – Silvercel® > \textbf{№ 7} Guanpolisept®
\end{itemize}

Table 2 – Effectiveness of antimicrobial biomaterials against clinical strains of \textit{K. pneumoniae}, \textit{A. baumannii} and \textit{P. aeruginosa} (mean values of ZOIs, M±mm)

<table>
<thead>
<tr>
<th>Biomaterials</th>
<th>Strains</th>
<th>\textit{K. pneumoniae} (n=11)</th>
<th>\textit{A. baumannii} (n=14)</th>
<th>\textit{P. aeruginosa} (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>№1 DCM</td>
<td>12.47±0.89</td>
<td>13.4±0.44</td>
<td>11.93±0.38</td>
<td></td>
</tr>
<tr>
<td>№2 DCM</td>
<td>11.52±0.69</td>
<td>13.91±0.28</td>
<td>12.57±0.35</td>
<td></td>
</tr>
<tr>
<td>№3 DCM</td>
<td>12.01±0.91</td>
<td>13.6±0.3</td>
<td>12.3±0.64</td>
<td></td>
</tr>
<tr>
<td>№4 Suprasorb®</td>
<td>13.49±0.7</td>
<td>14.26±0.2</td>
<td>8.51±0.47</td>
<td></td>
</tr>
<tr>
<td>№5 Silvercel®</td>
<td>6.68±0.13</td>
<td>6.45±0.16</td>
<td>6.32±0.11</td>
<td></td>
</tr>
<tr>
<td>№6 Urgotul®</td>
<td>8.03±0.28</td>
<td>7.38±0.28</td>
<td>7.58±0.2</td>
<td></td>
</tr>
<tr>
<td>№7 Guanpolisept®</td>
<td>10.99±0.75</td>
<td>10.13±0.27</td>
<td>6.25±0.11</td>
<td></td>
</tr>
<tr>
<td>№8 Bétadine®</td>
<td>9.69±0.27</td>
<td>8.27±0.27</td>
<td>9.52±0.14</td>
<td></td>
</tr>
</tbody>
</table>

Ranking of biomaterials by efficiency rating against clinical strains of \textit{P. aeruginosa} based on the average values of ZOIs (from the most effective):

\begin{itemize}
  \item \textbf{№ 2} DCM > \textbf{№ 3} DCM > \textbf{№ 1} DCM M > \textbf{№ 8} – Bétadine® > \textbf{№ 4} Suprasorb®
  \item \textbf{№ 6} Urgotul® > \textbf{№ 5} Silvercel® > \textbf{№ 7} Guanpolisept®
\end{itemize}

Samples with decamethoxime № 1-3 were the most effective against reference and clinical strains of \textit{P. aeruginosa}, their average values of ZOIs were not significantly different from each other (\textit{p>0.05}), and the effectiveness was equivalent, and the reliability of the difference in values with other biomaterials was equally high (\textit{p<0.001}).

For example, sample № 2 DCM against clinical strains of \textit{P. aeruginosa} was 1.48 times more effective than № 4 Suprasorb® (\textit{p<0.001}), 1.99 times more effective than Silvercel® (\textit{p<0.001}), 1.66 times more effective than Urgotul® (\textit{p<0.001}), 2.01 times – for Guanpolisept® (\textit{p<0.001}), 1.32 times – for Bétadine® (\textit{p<0.001}) (\textit{table 2, fig.}).

Biomaterial №8-Bétadine® was identified as the next most effective. The average diameters of ZOIs in relation to clinical strains of \textit{P. aeruginosa} for Bétadine® differed significantly from those for Suprasorb® by 1.12 times (\textit{p<0.05}), for Silvercel by 1.51 times (\textit{p<0.001}), for Urgotul® by 1.26 times (\textit{p<0.001}), for Guanpolisept® by 1.52 times (\textit{p<0.001}). Suprasorb® was 1.35 times more effective than Silvercel® (\textit{p<0.001}), 1.36 times more effective than Guanpolisept® (\textit{p<0.001}). The values of ZOIs for Suprasorb® and Urgotul® were not significantly different (\textit{p>0.05}). Urgotul® was 1.2 times more effective than Silvercel® (\textit{p<0.001}), 1.21 times more effective than Guanpolisept® (\textit{p<0.001}). Some clinical strains of

Figure – Zones of inhibition around samples of biomaterials №1-8.
Paeruginosa were completely resistant to Silvercel® and Guanpolisept®.

Thus, reference and clinical strains of Paeruginosa were most susceptible to antimicrobial biomaterials based on decamethoxine, as well as to Bétadine® biomaterial.

The prevention and treatment of healthcare-associated infections (HCAI) remains a global public health challenge [28].

Surgical site infections (SSIs), infectious complications of wounds and burns (including combat wounds) are mainly caused by microorganisms resistant to the most commonly used antimicrobial drugs and are characterized by multiple drug resistance. Carbapenem-resistant K.pneumoniae (CRKP), Paeruginosa (CRPA) and A.baumannii (CRAB) are among the dominant and threatening agents in the structure of pathogens [29-36].

New technologies and materials can help in this fight against HCAI, so the development of biomaterials with antibacterial properties is a promising area of research [37].

As a result of research and comparative assessment of antimicrobial properties of new and commercially available biomaterials based on antiseptics, a high level of antimicrobial activity of new biomaterials based on decamethoxine was revealed. The developed biomaterials were not inferior to modern effective wound dressings based on cationic detergents and silver-containing wound dressings, they actively inhibited the growth of reference and clinical strains of K.pneumoniae, A.baumannii, Paeruginosa, and were often the most active.

The results of the study showed that Suprasorb® and Guanpolisept®, which contain polyhexanide, have the highest activity against K.pneumoniae and A.baumannii among commercially available modern wound dressings; against Paeruginosa – Suprasorb®, and Bétadine® containing povidone-iodine. The effectiveness of silver-containing wound dressings in vitro was inferior to the effectiveness of dressings with cationic detergents and iodophors.

Giuomar, A. J. et al. also report high antibacterial activity of their developed and control commercial (Suprasorb®) polyhexanide-releasing membranes against K.pneumoniae, A.baumannii and Paeruginosa based on the disk diffusion test. The authors also note that Paeruginosa was the bacterial species that most often resisted the antibacterial activity of the polyhexanide-based biomaterials developed by the authors and commercial analogues. In one large-scale study, biomaterials loaded with 0.1% PHMB (polyhexamethylene biguanide) demonstrated antibacterial activity that exceeded that of a commercial silver-based wound dressing, but was sometimes inferior to equivalent membranes loaded with the antisepsics octenidine and povidone-iodine [38].

García, L. V. and co-authors found good antimicrobial properties of casein hydrogel dressings based on polyhexanide or Octiset® against S.aureus and Paeruginosa, and some samples with polyhexanide were more effective [39].

Eberlein, T et al found that dressings with PHMB removed the bacterial load significantly faster and better than dressings with silver in patients with locally infected or critically colonized wounds [40].

Stuermer, E. K, and others studied the antibiofilm activity of antimicrobial dressings, including silver-containing and polyhexanide-containing ones. The authors note that silver-containing wound dressings showed no bacteriostatic or bactericidal activity in a Paeruginosa biofilm model, whereas a polyhexanide dressing showed a significant inhibitory effect [41]. Dydak K, et al also found that dressings chemisorbed with polyhexanide or povidone-iodine provided equivalent or even higher antibiofilm activity than dressings containing silver molecules [42].

Conclusions.

Comparative studies of antimicrobial properties of developed biomaterials and commercially available antimicrobial dressings revealed high antimicrobial properties of new decamethoxin-based biomaterials against reference and clinical strains of target microorganisms, which are leading pathogens of wounds and burns.

Reference and clinical strains of A.baumannii show the greatest susceptibility to samples with decamethoxine, as well as to biomaterials Suprasorb® X + PHMB and Guanpolisept® based on polyhexanide.

Biomaterials with decamethoxin Ne1, Ne2 and Ne3, Suprasorb®, Guanpolisept® and Bétadine® were determined to be the most effective against reference and clinical strains of K.pneumoniae.

Reference and clinical strains of Paeruginosa are most susceptible to biomaterials with decamethoxin Ne1, Ne2 and Ne3 and Bétadine®. Prospects for further research.

In the future, we plan to adapt this biomaterial composition to different physical forms of wound dressings. The developed biomaterials are presented in the form of a film. These polymer films, made by casting from a solvent, are well suited as a base layer in multilayer compositions, hydrogels, plasters. Hydrogel alginites are also very useful in lyophilized form. We plan to expand the range of polymers that serve as a matrix for the controlled release of decamethoxine (for example, cellulose, chitosan, hyaluronic acid), and to investigate the microbiological and physicochemical parameters of new compositions. In order to increase the efficiency of the system with controlled release of the active substance, it is planned to investigate the preprogramming of the system (biomaterial) using the combination of physical and chemical techniques.

References


Різноманітність штамів бактерій, включаючи пронизані життєво важливими для медицини інфекційними штамами, вимагає пошуків нових стратегій боротьби з ними. Антимікробні властивості біоматеріалів, розроблених на основі декаметоксину, були вивчені в контексті антибактеріальних досліджень, проведених в нашій роботі. Результати показали, що біоматеріали із декаметоксином демонструють високу ефективність проти широкого спектра цільових мікроорганізмів.

**Антимікробні властивості** нових біоматеріалів із декаметоксином (ДКМ №1-3) та комерційно доступних біоматеріалів і зсувиць (це розглядалося в попередніх дослідженнях), показали, що біоматеріали із декаметоксином №1, №2 та №3 демонструють найвищу ефективність в боротьбі з розсіяніми штами K.pneumoniae, A. baumannii та P. aeruginosa.

**Порівняльне дослідження** антимікробних властивостей біоматеріалів та пов’язок на основі антисептиків, що відносяться до грамнегативних бактерій, є дуже актуальною і перспективною. Розроблені біоматеріали з декаметоксином не поступалися сучасним ефективним рановим покриттям і зсувиць, які використовуються в практиці лікування ран і інфекцій. Нові біоматеріали, розроблені на основі декаметоксину, можуть бути використані в лікуванні тяжких інфекційних захворювань, що вимагають подальших досліджень.

**Ключові слова:** антимікробні біоматеріали, K.pneumoniae, A. baumannii, P. aeruginosa, антисептики, декаметоксин.
Mикробіологія / Microbiology

ere and clinical strains of target microorganisms, which are leading pathogens of wounds and burns. Reference and clinical strains of A. baumannii show the greatest susceptibility to samples with decamethoxin (№ 1-3 DCM), as well as to biomaterials Suprasorb® and Guanpolisept® based on polyhexanide. Biomaterials with decamethoxin №1, №2 and №3, Suprasorb®, Guanpolisept® and Бétadine® were determined to be the most effective against reference and clinical strains of K. pneumoniae. Reference and clinical strains of P. aeruginosa are most susceptible to biomaterials with decamethoxin №1, №2 and №3 and Бétadine®.

Conclusions. The developed biomaterials were not inferior to modern effective wound dressings based on cationic detergents and silver-containing wound dressings, they actively inhibited the growth of reference and clinical strains of K. pneumoniae, A. baumannii, P. aeruginosa, and were often the most active. The effectiveness of silver-containing wound dressings in vitro was inferior to the effectiveness of dressings with cationic detergents and iodos-phors.

Key words: antimicrobial biomaterials, K. pneumoniae, A. baumannii, P. aeruginosa, antiseptics.

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A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article.

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Drehval O. A., Lesychna A. V., Drehval I. V., Sklyar T. V.
INFLUENCE OF CARBON AND NITROGEN SOURCES ON BIOMASS YIELD AND FUNGISTATIC ACTIVITY OF TRICHODERMA VIRIDE KMB-F-15
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Micromycetes of the Trichoderma genus are the most common biological agents used in agriculture today to control plant mycoses. Using biological products allows us to produce environmentally friendly agricultural products and reduce the chemical burden on the environment. When choosing carbon and nitrogen sources as a basic base for developing the optimal composition of the nutrient medium for deep cultivation of microorganisms, it is necessary to consider their genus and strain characteristics.

In this study, we investigated the effect of carbon and nitrogen sources on the biomass accumulation and fungistatic activity of Trichoderma viride strain KMB-F-15, an antagonist of a wide range of phytopathogenic fungi. The fungistatic activity was determined by inhibition of growth of the phytopathogenic fungus Fusarium culmorum IMBF-50716 when the filtrate of T. viride KMB-F-1 culture fluid was added to the dense medium. It was found that glyco- erol and green molasses at a concentration of 20 g/l resulted in the highest yield of dry biomass of T. viride KMB-F-15 (5.0 g/l and 4.9 g/l, respectively). The most favourable nitrogen sources for the fungus growth at a concentration of 5 g/l were yeast autolysate (dry biomass yield – 4.4 g/l) and ammonium chloride (3.3 g/l). The fungistatic activity of T. viride KMB-F-15, regardless of the carbon source, was high (94.7-100%). The manifestation of fungistatic activity was influenced by the source of nitrogen nutrition. The highest percentage of growth inhibition of the phytopatho- gen was observed when corn extract, yeast autolysate, L-glutamic acid, ammonium chloride or ammonium sulfate were used (88.5-100%).

Key words: Trichoderma, submerged cultivation, accumulation of biomass, antagonistic properties, phytopatho- genic fungi.

Connection of publication with planned research works.

The article is a fragment of the research work of the Department of Microbiology, Virology and Biotechnol- ogy of Oles Honchar Dnipro National University: “Antagonistic and synergistic relationships in microbial associations” (state registration number 0122U001456).

Introduction.

Today, the chemical method prevails in protecting agricultural plants from pests. Compared to other pesti- cides, fungicides are considered less threatening to non- target organisms, but some are also banned as evidence of their negative impact on biota is accumulated [1]. In addition, the widespread use of fungicides creates se-