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EFFECT OF CARBON DIOXIDE HOP EXTRACT ON GROWTH INTENSITY AND ANTIBIOTIC RESISTANCE LEVEL OF THE TRANSFORMANT STRAIN KLEBSIELLA PNEUMONIAE

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The effect of hop carbon dioxide extract on the growth and ampicillin resistance of the museum strain *Klebsiella pneumoniae* 5055 with an experimentally acquired beta-lactam antibiotic resistance plasmid from *Escherichia coli* "D"strain has been studied. The transfer of the antibiotic resistance plasmid to the specified strain has been confirmed by the method of horizontal gel electrophoresis, real-time polymerase chain reaction and when studying the antibiotic sensitivity profile by phenotypic methods. It was found that hop carbon dioxide extract produced a pronounced antimicrobial effect on the transformant strain of *Klebsiella pneumoniae*, but did not affect the level of its resistance to ampicillin. After analyzing the data of our studies, it can be assumed that the inhibitory effect of the carbonic acid hop extract on the growth of bacteria was not accompanied by an antiplasmid effect, since resistance to beta-lactams in the *Klebsiella pneumoniae* 5055+pECD strain was associated precisely with the acquisition of an ampicillin resistance plasmid by in vitro transformation.

Key words: carbonic acid hop extract, transformant strain of *Klebsiella pneumoniae*, antimicrobial activity, antiplasmid effect.

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ВПЛИВ ЕКСТРАКТУ ХМЕЛЮ ВУГЛЕКИСЛОТНОГО НА ІНТЕНСИВНІСТЬ РОСТУ ТА РІВЕНЬ АНТИБІОТИКОРЕЗИСТЕНТНОСТІ ТРАНСФОРМАНТНОГО ШТАМУ KLEBSIELLA PNEUMONIAE

Досліджено вплив екстракту хмелю вуглекислотного на ріст та стійкість до ампіциліну музейного штаму *Klebsiella pneumoniae* 5055 з набутою експериментальним шляхом плазмідою резистентності до бета-лактамних антибіотиків від штаму *Escherichia coli* "D". Перенос плазміди антибіотикорезистентності зазначеному штаму підтверджено методом горизонтального гель-електрофорезу, полімеразної ланцюгової реакції у реальному часі та при дослідженні профілю антибіотикочутливості фенотиповими методами. Встановлено, що екстракт хмелю вуглекислотного створював виражену протимікробну дію щодо трансформантного штаму *Klebsiella pneumoniae,* проте не впливав на рівень його резистентності до ампіциліну. Проаналізувавши дані наших досліджень можна зробити припущення, що інгібуюча дія екстракту хмелю вуглекислотного на ріст бактерій не супроводжувалась антиплазмідним ефектом, оскільки стійкість до бета-лактамів у штаму *Klebsiella pneumoniae* 5055+pECD була пов'язана саме з отриманням шляхом трансформації *in vitro* плазміди резистентності до ампіциліну.

Ключові слова: екстракт хмелю вуглекислотний, трансформантний штам *Klebsiella pneumoniae*, протимікробна активність, антиплазмідна дія.

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The spread of resistance among the most common bacterial pathogens is recognized as a global challenge for the healthcare industry worldwide, therefore the relevance and seriousness of this problem is fully recognized by the international medical community. In 2017, WHO first published, and in 2024 updated, a list of bacteria that pose the greatest threat to human health. The list classifies bacteria into three priority categories: critical, high, and medium priority, according to the urgency of developing new approaches to combat these pathogens. This list also includes one of the leading pathogens of nosocomial infections, *Klebsiella pneumoniae* (critical priority level). Antibiotic-resistant strains of *Klebsiella pneumoniae* can cause severe and often fatal infectious diseases, such as bloodstream infections, hospital-acquired pneumonia, etc. [2, 7, 19].

Due to the significant spread of resistance of pathogens of purulent-inflammatory infections to a wide range of antibiotics, there is a need to find and introduce alternative drugs into clinical practice. Therefore, in recent years, the number of studies aimed at isolating and identifying new biologically active substances from plants has increased active chemicals with existing antibacterial properties, which will allow to expand the arsenal of antimicrobial agents and reduce the risks of the formation and spread of antibiotic resistance [1, 5].

One of the promising plants for the development of drugs with antimicrobial action is hops. Biologically active ingredients of hops can be potential antibiotic compounds that, independently or in combination with other antibacterial substances, open new ways to combat infections caused by pathogenic, including multidrug-resistant bacteria [12, 13]. The molecular mechanisms of the antibacterial effects of hops are based, first of all, on the damaging effect of the biologically active ingredients lupulone and humulone on the bacterial cell membrane, as well as the inhibition of redox processes, protein synthesis, DNA and RNA [8].

In addition, it has been established that some natural product extracts and their derivatives can inhibit the formation and spread of antibiotic resistance among strains of infectious diseases by inhibiting conjugation as a way of transferring plasmids from strains carrying antibiotic resistance genes to recipient strains, and such a biological compound as rottlerin, a specific protein kinase inhibitor, can become the basis for further research into substances with effective antiplasmid activity [3]. Unfortunately, the outlined principles for overcoming infections caused by multidrug-resistant pathogens have not yet gained practical significance, since there is insufficient data on the results of "antiplasmid treatment" carried out in vivo, even in experimental models [4].

The purpose of the study was to establish the effect of carbonic hop extract on the growth and ampicillin resistance of a *Klebsiella pneumoniae* strain with an experimentally acquired plasmid of resistance to beta-lactam antibiotics.

Materials and methods. The studies have been conducted on the basis of the State Institution "Institute of Microbiology and Immunology named after I.I. Mechnikov of the National Academy of Medical Science of Ukraine" from May to August 2025. The object of the study was the museum strain *Klebsiella pneumoniae* 5055 from the collection of microorganisms of the State Institution "Institute of Microbiology and Immunology named after I.I. Mechnikov of the National Academy of Medical Science of Ukraine", to which a plasmid of resistance to beta-lactams from the antibiotic-resistant strain *Escherichia coli* "D" was experimentally transferred by horizontal transfer (by simple transformation), a carrier of genes of resistance to beta-lactam antibiotics *bla*TEM, *bla*SHV and *bla*CTX-M. Plasmid DNA was obtained from the donor strain Escherichia coli "D" by alkaline lysis with phenol extraction according to Kado and Liu [9]. Plasmid isolation was carried out by horizontal gel electrophoresis using a PG9 electrophoresis chamber ("Dia-M LLC"), a PEF-3 electrophoresis device ("Medtekhnika"), an agarose gel of medium electroendoosmosis (Khimlaborreaktiv LLC) at an electric field voltage of 4-6 Volts. The approximate size of the detected plasmid was determined by the criterion of its electrophoretic mobility [18]. As an additional marker of the size of the detected plasmids, DNA of phage lambda digested with EcoRV restriction enzyme (Laboratory of Leading Biotechnologies "NEOGEN") was used. Obtaining competent cells of the recipient strain *Klebsiella pneumoniae* 5055 and interspecific transformation with plasmid DNA were carried out according to the method of Ledenberg and Cohen [16]. The efficiency of transfer of genes of resistance to β-lactam antibiotics was confirmed by real-time polymerase chain reaction (PCR-RF) using a primer system [6] produced by the "Fermentas" enterprise. The interspecific transfer of resistance to beta-lactams from the donor strain *E. coli* to the recipient strain *K. pneumoniae* was also confirmed by studying antibiotic sensitivity profiles by phenotypic methods. The studied transformant strain *Klebsiella pneumoniae* 5055+pECD was cultivated at 35 ºC for 24 h in Mueller-Hinton broth and in Mueller-Hinton broth with the addition of hop carbon dioxide extract at a subinhibitory concentration (1 mg/ml), inoculations were carried out from serial dilutions on Mueller-Hinton agar and after 24-hour cultivation of the cultures at 37 ºC, the number of grown colonies was counted. To study the possible effect of hop carbon dioxide extract on the antibiotic resistance of the transformant strain *Klebsiella pneumoniae* 5055+pECD, the grown colonies were replicated on Mueller-Hinton agar with ampicillin at a concentration of 50 µg/ml, the cultures were grown for 24 h at a temperature of 35 ºC.

Results of the study. According to the results of the studies, the transformant strain *Klebsiella pneumoniae* 5055+pECD was obtained by interspecific transfer of antibiotic resistance. In the specified strain, the presence of a plasmid with dimensions that coincide with the dimensions of the plasmid of the donor strain was established by horizontal gel electrophoresis, and genes for resistance to beta-lactam antibiotics were also detected by real-time polymerase chain reaction. Interspecific transfer of resistance to beta-lactams from the donor strain *Escherichia coli* "D" to the recipient strain *Klebsiella pneumoniae* 5055 was also confirmed by studying antibiotic susceptibility profiles by phenotypic methods.

When cultivating the transformant strain in Mueller-Hinton broth and subsequent plating on Mueller-Hinton agar (control sample), the number of colony-forming units was 3.6 x 10^8 CFU/ml. When cultivating *Klebsiella pneumoniae* 5055+pECD in Mueller-Hinton broth with the addition of hop carbon dioxide extract in a subinhibitory concentration and subsequent plating on Mueller-Hinton agar, the number of colony-forming units was 4.9 x 10^7 CFU/ml, i.e., inhibition of culture growth by 86.4 % was observed compared to control values. Fig. 1 shows the pronounced antimicrobial effect of carbonic hop extract on the studied transformant strain.

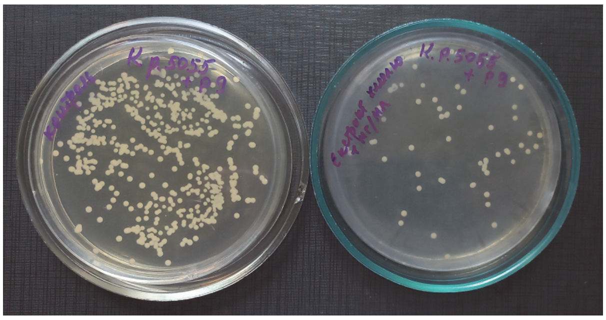

Fig. 1. Effect of ECV on the growth properties of the transformant strain *Klebsiella pneumoniae* 5055+pECD.

The next stage of our study was to study the effect of carbonic acid hop extract on the antibiotic resistance of the transformant strain – the carrier of the beta-lactam resistance plasmid. After cultivating the studied strain in the presence of hop carbon dioxide extract, when plating on a medium without antibiotics, 67 colony-forming units were obtained. All colonies after replication on ampicillin medium retained the ability to grow (Fig. 2), i.e., cultivation of the studied strain *Klebsiella pneumoniae* 5055+pECD in a medium with a subinhibitory concentration of carbonic hop extract did not affect the level of its resistance to ampicillin.

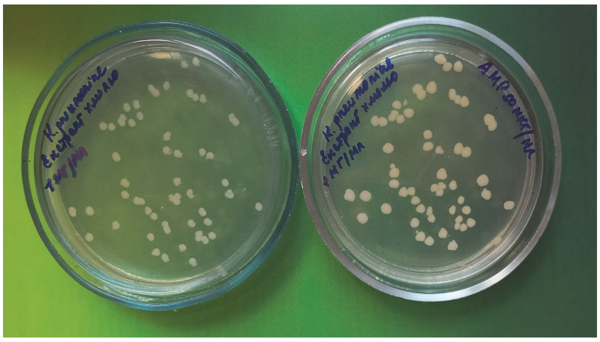

Fig. 2. Results of studying the effect of ECV on the resistance of the transformant strain *Klebsiella pneumoniae* 5055+pECD to ampicillin.

Discussion. Analysis of literature sources showed that in recent years a number of studies have been conducted that also confirm the antimicrobial efficacy hop derivatives against microorganisms of different taxonomic groups. According to Piasecki B et al, extracts and essential oils from Jung's hop samples showed antimicrobial activity against 8 strains of microorganisms pathogenic to humans, including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci. Moreover, hop extracts were significantly more active than essential oils, and their activity correlated with a high concentration of xanthohumol [17].

Studies conducted using strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa, Candida albicans* also revealed antibacterial properties of hop extracts and proved the slow formation of resistance to them in these pathogens [11].

Significant antimicrobial activity of ethanol extracts of five hop varieties was confirmed on the *Bacillus subtilis* strain. The authors conducted metabolome profiling of hop extracts and found that the antimicrobial activity of hop extracts is mainly due to the metabolites colupulone and lupulon [15]. High antimicrobial activity of the purified fraction of another metabolite of hop extract – xanthohumol, was found on strains of *Staphylococcus aureus* and *Lactobacillus acidophilus*. [14].

Hop extracts can not only create an antibacterial effect, but also enhance the effect of other antimicrobial agents. Khaliullina A. et al, established a synergistic effect of hop extract with aminoglycoside, fluoroquinolone and cephalosporin antibiotics against pathogens of purulent-inflammatory diseases of the oral cavity, which allowed to reduce the required concentration of antimicrobial agents and thus reduce the risk of both side effects and the development of bacterial resistance [10].

Thus, today the antimicrobial activity of hops has been confirmed by numerous experimental works of domestic and foreign researchers. Scientific reports highlight not only the degree, but also the mechanisms of antimicrobial action of hop derivatives against various types of microorganisms. In further studies on the biological properties of hops, in our opinion, it would be important to clarify the influence of hop metabolites on antibiotic resistance in microorganisms of different taxonomic groups in order to find ways to effectively combat the spread of antibiotic resistance.

Limitations. A limitation of this study is that the study of the effect of hop carbon dioxide extract was carried out only on one type of transformant strain – *Klebsiella pneumoniae*, therefore, the results of the study will be incorrectly extrapolated to other types of enterobacteria.

Conclusion

It was established that hop carbon dioxide extract produced a pronounced antimicrobial effect on the transformant strain *Klebsiella pneumoniae* 5055+pECD, obtained by simple transformation – horizontal transfer of an antibiotic resistance plasmid from an ampicillin-resistant strain of *E. coli* to an ampicillin-sensitive strain of *Klebsiella pneumoniae*. However, hop carbon dioxide extract did not cause the loss of resistance to beta-lactam antibiotics in the transformant strain. Since resistance to beta-lactams in the studied strain *Klebsiella pneumoniae* 5055+pECD was associated with the acquisition of the pECD plasmid by in vitro transformation, it can be assumed that the inhibitory effect of carbonic hop extract on bacterial growth is not accompanied by an antiplasmid effect.

Prospects for further research. We believe that studies investigating the effect of hop extracts on transformant strains of enterobacteria of various species, as well as on the processes of horizontal transfer of antibiotic resistance genes, are promising, since hop compounds (such as lupulone) act as ionophores that dissipate the transmembrane proton gradient of bacterial cells and can inhibit the conjugation process, the main mechanism by which bacteria exchange resistance plasmids.

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Conflict of interest. The authors have no conflicts of interest to declare.

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