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OGÓLNOPOLSKA KONFERENCJA NAUKOWA "POD MIKROSKOPEM" IV EDYCJA "DROBNOUSTROJE CODZIENNYM WYZWANIEM DLA MIKROBIOLOGA"

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Konferencja pod Honorowym Patronatem
JM Rektora Uniwersytetu Medycznego
im. Karola Marcinkowskiego w Poznaniu

Poznań, 12 marzec 2018

ORGANIZATOR

Katedra i Zakład Genetyki i Mikrobiologii Farmaceutycznej
Uniwersytetu Medycznego im. Karola Marcinkowskiego w Poznaniu

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PROGRAM

8.00 REJESTRACJA UCZESTNIKÓW

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Prof. dr hab. Eugenia Gospodarek-Komkowska
Katedra i Zakład Mikrobiologii Collegium Medicum im. L. Rydygiera w Bydgoszczy, Uniwersytet Mikołaja Kopernika w Toruniu

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Dr hab. Małgorzata Leszczyńska
Katedra Otolaryngologii, Chirurgii Głowy i Szyi oraz Onkologii Laryngologicznej, Uniwersytet Medyczny im. K. Marcinkowskiego w Poznaniu

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Dr Alicja Kuch
Krajowy Ośrodek Referencyjny ds. Diagnostyki Bakteryjnych Zakażeń Ośrodkowego Układu Nerwowego

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Krajowy Ośrodek Referencyjny ds. Lekowrażliwości Drobnoustrojów

12.00 „Dlaczego czasami antybiotyki nie działają?..."
Dr Hanna Tomczak
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Mgr Marcin Brzozowski
bioMérieux Polska Sp. z o.o.

12.45 PRZERWA KAWOWA

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Mgr Ewa Kaniuk
Kierownik Produktu, Argenta Sp. z o.o. Sp. k.

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Dr Bogusław Grabowski
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Dr hab. Marcin Schmidt

Katedra Biotechnologii i Mikrobiologii Żywności, Uniwersytet Przyrodniczy w Poznaniu

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Dr hab. Anna Sip

Katedra Biotechnologii i Mikrobiologii Żywności, Uniwersytet Przyrodniczy w Poznaniu

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Dr Małgorzata Prażyńska

Katedra i Zakład Mikrobiologii Collegium Medicum im. L. Rydygiera w Bydgoszczy, Uniwersytet Mikołaja Kopernika w Toruniu

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Katedra Biotechnologii i Mikrobiologii Żywności, Uniwersytet Przyrodniczy w Poznaniu

16.00 „Znane strategie - nowe wyzwania”

Dr Jolanta Długaszewska

Katedra i Zakład Genetyki i Mikrobiologii Farmaceutycznej, Uniwersytet Medyczny im. K. Marcinkowskiego w Poznaniu

16.15 „Quorum quenching - strategia zapobiegania tworzeniu się biofilmu i psuciu się żywności przez spp.”

Dr hab. inż. Kamila Myszka

Katedra Biotechnologii i Mikrobiologii Żywności, Uniwersytet Przyrodniczy w Poznaniu

16.45 ZAKOŃCZENIE KONFERENCJI I WRĘCZENIE CERTYFIKATÓW

MICROBIAL QUALITY OF SPROUTS - CHALLENGE FOR THE MICROBIOLOGIST

Anna Dobrowolska[1]*, Joanna Szulc[2], Agnieszka Musiałowska[1], Agnieszka Wita[1], Katarzyna Czaczyk[1]

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Sprouted seeds are very attractive component of the human diet due to content of easily absorbed vitamins and microelements. This is particularly important for vegetarians and vegans. However, not all consumers are aware that sprouts are one of the most microbiologically contaminated food product. The total count of bacteria in fresh sprouts achieved the high level - up to 10^7-10^9 cfu/g and presence of pathogenic bacteria is also observed (*Salmonella* sp., *Listeria monocytogenes*). Fenugreek sprouts, produced on ecological farm in Germany, were the reason of large outbreak of the hemolytic–uremic syndrome caused by *Escherichia coli* O104:H4 occurred in Europe in May 2011. Despite many studies on possibility of disinfection seeds after germination, have not been found effective method of elimination foodborne pathogens from both seeds and sprouts.

The aim of this study was the possibility of using aqueous solutions of chloride dioxide (up to 1000 ppm) to improve microbiological quality of seeds and fresh sprouts.

Radish seeds (Opolanka variety) and broccoli (Calabrese Natalino variety) and sprouts produced from these seeds were experimental material in this study. The following microbiological analysis have been studied: total aerobic bacteria count (PN-EN ISO 4833: 2004), total yeast and molds count (PN-ISO 21527-1:2009P), the number of Enterobacteriaceae (PN-ISO 21528-2), the presence of *Salmonella* sp. (PN-ISO 6579:2003/A1:2007P) and *Listeria monocytogenes* (PN-ISO 11290:1:1999/ A1:2005P).

The obtained results indicated that the chloride dioxide aqueous solutions (800-1000 ppm) have significant effect on the reduction in the number of examined groups of microorganisms in seeds. Despite this, the total aerobic bacteria count in sprouts produced from these seeds was high 10^6-10^7cfu/g. The combination of two methods: seed disinfection using chloride dioxide aqueous solutions and the use low concentrations of chloride dioxide aqueous solutions during the germinations (25, 50 ppm) allowed for significant reduction of total plate count.

CURRENT AND FUTURE TRENDS IN BIOFILM DIAGNOSTICS

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Introduction: Biofilm is complex, multi-layered community of microbes embedded within extracellular matrix, which alters their metabolism and protects them from chemotherapeutic agents. It results in persistence of microorganisms and spread of hard-to-heal infections. Biofilm is able not only to colonize biomedical implants, including artificial heart valves, endoprostheses, bone wrenches, stents and catheters, but also to colonize virtually every part or organ of patient's body. Diagnostics of biofilms dispose many challenges due to complex process of their life cycle, therefore search for new methods of their detection is a need of paramount importance.

Review: In the present work a spectrum of present and future methods of biofilm diagnostics is discussed. Apart from simple but not precise biochemical methods, modern methods involving advanced measurement equipment are presented, including: isothermal microcalorimetry, confocal microscopy, use of X-rays, coherence tomography, luminometry, impedance spectrometry as well as methods based on metabolomics, proteomics and metagenomics approach.

Conclusions: The research and development of new, accurate methods of biofilm diagnostics is urgently needed to limit health consequences related with presence of this microbial structure.

Key words: biofilm, diagnostics, infections, microcalorimetry, confocal microscopy, X-rays, coherence tomography, metabolomics, proteomics, metagenomics.

OLD STRATEGIES - NEW CHALLENGES

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Bacterial resistance to antimicrobials is an increasing threat to public health. The majority of current infectious diseases are almost untreatable by conventional antibiotic therapy due to the appearance of multidrug-resistant bacteria. Therefore, efforts are being made to develop new therapeutic strategies to combat drug-resistant bacteria. Natural plant products, nanomaterials, and phototoxic compounds have proven to be outstanding candidates for these much-needed therapeutics.
People from antiquity have used botanical products. Plants can produce a rich variety of antimicrobial secondary metabolites (phytochemicals). Some metabolites represent constitutive chemical barriers to microbes (phytoanticipins) others represent inducible antimicrobials (phytoalexins). Multiple classes of these products, including terpenoids, saponosides, polyphenols, glucosinolates, flavonoids have been described. The bioactivities many of them were investigated and their use as “antibiotic potentiators” or “virulence attenuators” it seems to be promising.

In the 21st century, we observe the progress in nanotechnology. Some metals, such as zinc, silver, and copper, exhibit antibacterial activity in their bulk form but the implementation of nanotechnology has further enhanced their antibacterial properties. Other materials, such as iron oxide, are not antibacterial in their bulk form but may exhibit antibacterial properties in nanoparticulate form. Another aspect concerns nanomaterials is nanopharmacology. Different nanomaterials such as magnetic nanoparticles, polymers, liposomes, micelles, emulsions, dendrimers, fullerenes, quantum dots and carbon nanotubes have been investigated for drug delivery applications.
Many ancient civilizations utilized light in the treatment of disease, but it was not until early last century that phototherapy reappeared. Currently, photodynamic therapy (PDT) used involves the administration of a photosensitizing agent followed by exposure to visible light, and cytotoxic reactive oxygen species are produced resulting in cell death. As photosensitizers, porphyrins, tetrapyrroles, phthalocyanines, porphycenes, texaphyrins and sapphires are used. Importantly, no PDT-resistant microorganisms have been found so far.

The use of these alternative strategies can be among the most promising approaches to reducing the use of antibiotics and can help to solve the antibiotic-resistance problem.

HIGH FREQUENCY OF MACROPHAGES EXPRESSING ELEVATED LEVEL OF CD80, PD-LS AND TLR1 IN NASAL POLYPS OF CRS PATIENTS

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Background: Identification of the association between tissue biomarkers, their surrogates in blood and clinical features, could provide new diagnostic tools and facilitate adequate choices of therapeutic interventions for selected patients suffering from CRS.

Objective: The aim of present study was the assessment of macrophages in the polyp tissue and monocytes in the peripheral blood in the course of CRSwNP, and their functional immunophenotype.

Methods: We analyzed 31 patients with CRSwNP. Nasal mucosa tissue was obtained via functional endoscopic sinus surgery (FESS). The control group included 10 patients with deviated nasal septum (DNS). Fluorochrome stained cells were proceed to acquisition using FACS Canto flow cytometer (BD Biosciences), and the results were analyzed using the software FACS Diva (BD Biosciences). The percentage of positive cells and mean fluorescent intensity (MFI) were assessed.

Results: In our study, we observed a significantly higher level of CD80, CD274, CD273 and TLR1 in nasal polyps compared to blood samples from patients with CRSwNP. This finding may suggest the importance of the PD-1 pathway as a therapeutic target in CRS and an important role for TLR1 in nasal polyp formation and maintenance.

Conclusions: Our results may provide some insight into potential future targets of recurrent nasal polyp treatment and contribute to a better understanding of the inflammatory process in chronic rhinosinusitis.

SENSITIVITY ON ANTIBIOTICS AND CHEMOTERAPEUTIC AGENTS OF THE MOST COMMONLY ISOLATED GERM FROM URINARY SYSTEM'S INFECTIONS IN AMBULATORY PATIENTS

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The urinary system's infections represent 10-20% out-of-hospital bacterial infections among people. The most common treatment has a form of empirical therapy and the most frequently applied medicines are: co-trimoxazole, ciprofloxacin, nitrofurantoin, amoxicillin with clavulanic acid. A possibility of multiple drug resistant strains' isolation should also be taken into account while choosing a medicine.

It is important to determine the main etiological factor of infections and define its sensitivity on medicines applied in empirical therapy of urinary system's infections.

The strains were analysed in 01.01.2017-31.12.2017. For analysis were submitted 1153 isolates from urine cultures of ambulatory patients, where a quantity indicated a significant bacteriuria. The isolation of the strains was performed on selective-diffrentiating medium and chromogenic ones. The identification was held with help of biochemical tests. Drug sensitivity was defined by a disc-diffusion method.

The biggest percentage of the isolates i.e 83% was represented by rods of Enterobacteriacae family, 13,5% cocci Gram positive and only 2% by non-fermentic rods. Among the rods of Enterobacteriacae family the most dominating was *Escherichia coli* - 73%, where 4,4% of them were strains of *E. coli* with ESBL positive phenotype of refractoriness, bacterial multiple drug resistant strains.

Sensitivity of *E. coli* strains used in empirical therapy of urinary system's infections was following: co-trimoxazole - 73,73%, nitrofurantoin - 93,19%, fosfomycinum - 100%, ciprofloxacin - 74,87%, amoxicillin with clavulanic acid - 91,38%. Sensitivity for ampicillin and amoxicillin was obtained in approx. 50% of cases.

Sensitivity of *E. coli* strains with ESBL positive type of refractoriness against medicines applied in empirical therapy, was much smaller in case of co-trimoxazole, ciprofloxacin, amoxicillin with clavulanic acid and consequently came up to 38,7%, 23,3% and 70,9%. At the same time sensitivity for fosfomycinum came up to 100%, and for nitrofurantoin - 90%.

The main etiological factor of urinary system's infections among ambulatory patients is *Escherichia coli*. Obtained sensitivity among *E. coli* strains without mechanism of resistants, confirms validity of applying co-trimoxazole, nitrofuran derivatives, ciprofloxacin, fosfomycinum and amoxicillin with clavulanic acid in empirical therapy of urinary system's infections. Due to low sensitivity of *E. coli* for ampicillin/amoxicillin, it is not advisable to apply these medicines empirically. Medicines which showed high sensitivity on both *E. coli* with mechanism of resistants and without mechanism of resistants - nitrofurantoin and fosfomycinum deserve special attention.

Occurence of ESBL positive strains and bacterial multiple drug resistant strains causing urinary system's infections, where the spectre of applied medicines can be very limited, advocates a need of performing urine cultures and determining cultured germs' drug resistance, especially at patients who had been hospitalised earlier or received antibiotics lastingly.

The study and coverage of the results were held due to courtesy of DIAGNOSTYKA Sp. z o.o.

BRIEFLY ABOUT MYELOCYTOMATOSIS VIRUSES AND *MYC* GENES

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Avian myelocytomatosis virus Mc29 was isolated in Bulgaria in 1961 from a Rhode Island Red chicken and belongs to the group of acute avian leukosis retroviruses. All three genes essential for the replication of retroviruses, i.e. gag, pol and env, are defective in Mc29 virus and there is a need for a helper virus for its replication.

In vitro the virus Mc29 transforms fibroblasts, epithelial cells and macrophages. Its i*n vivo* transmission causes primarily myelocytomatosis and myelocytomas in chickens, guinea fowl and Japanese quail while haemocytoblastosis predominates in turkey. The virus is also responsible for cancer growths in kidney, pancreas, liver, thymus.

Virus Mc29 expresses a specific oncogene *v-myc* as a part of gag-myc fusion protein (gag fusion part is unnecessary for its transformation ability). Later, v-myc oncogene was identified in some other retroviruses (CM2, OK10, MH2, FH3, Mc31, some J viruses such as strain 966) that belong to the group of myelocytomatosis viruses, the prototype of which is virus Mc29. They induce a broad range of neoplastic diseases (carcinomas, endotheliomas, mesotheliomas, sarcomas, myelocytomatosis) in their avian hosts. The MH2 virus expresses both v-myc and v-myl, which can cooperate in transformation *in vitro* and *in vivo*.

Cellular homologues of *v-myc* (*c-myc, L-myc, N-myc*) have been observed in avian as well as mammalian genomes. Myc is a transcription factor of the basic-helix-loop-helix-leucine zipper (BHLH-LZ) family that positively or negatively regulates expression of thousands target genes influencing key biological functions such as protein synthesis, glucose metabolism, cell proliferation and differentiation, apoptosis. Myc modulates different micro RNAs, majority of which are repressed.

The disturbed regulation of *myc* gene, that can be a result of chromosome translocation, amplification/overexpression or point mutations, has been suggested to be involved in pathogenesis of a wide variety of human and animal cancers.

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MICROARRAY TECHNIQUE: AN ADVANCED PAN-GENOMIC DIAGNOSTIC TOOL

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In contrast to yesterday, today there are wide ranges of diagnostic tools, which can be utilized in different microbiology laboratories. The number of referring patients, clinical samples, lab financial budget and the level of related laboratory, determine the types of diagnostic methods. Normally, the nucleic acid based technologies such as Polymerase Chain Reactions (PCRs) are commonly used as proper, cost effective, sensitive and specific tools. The PCRs are effective techniques for limited samples; however, they are not suitable and cost effective when there are a huge number of specimens. By the increase of technologies, there are some powerful techniques which can be used when the number of samples is high. Among different options, the advanced pan-genomic technology of Microarray can be introduced as an acceptable choice when the number of specimens is huge. In due to the target molecule, the microarray technology is divided into three main techniques of DNA, RNA and Protein microarrays. The utilization of microarray is recommended for an accurate, rapid, specific, sensitive, reliable and flexible diagnostic procedure. It is also cost effective for huge number of specimens.

Indeed, the microarray technology is a combination of bioinformatics, molecular biology and computer sciences. For this reason, the microarray technique is composed of dry (*in silico* procedure) and wet (*in vitro* procedure) labs. Thus, the employing of a bioinformatician in the presence of microarray tool is a must and recommended. A skillful bioinformatician is able to decrease the probable biases and provide us a sharp and accurate outcome by this lab-on-chip technology. The chip may include different types of microorganisms - from opportunistic to pathogenic microbial agents such as Uropathogenic *Escherichia coli* (UPEC), *Candida albicans*, *Klebsiella pneumoniae*, *Helicobacter pylori*, etc. The application of effective and powerful diagnostic tools results in definite diagnosis and treatment.

COMPARISON OF ANTIBIOTIC RESISTANCE OF *HELICOBACTER PYLORI* IN IRAN AND POLAND

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Helicobacter pylori is a Gram-negative, spiral-shaped bacterium that infects the gastric mucosa of more than half human population in the world. *H. pylori* can cause gastritis, peptic ulcer disease, and gastric cancer. The aim of this study was a comparison of *H. pylori* antibiotic resistance in Iran and Poland. Electronic databases including PubMed-Medline, Scopus, and Google Scholar were chosen to find published articles from the last 10 years. The medical terms including "*H. pylori*" AND "resistance" AND "Iran" or "Poland" were selected as related keywords. Following evaluation of titles, abstracts, and full texts, 23 relevant articles were selected and included in this study. For statistical calculation was used Mann-Whitney test. They were taken into account 5 antibiotics: metronidazole (MTZ), amoxicillin (AMX), clarithromycin (CL), tetracycline (TC), and levofloxacin (LE).

From Iran were included 1253 *H. pylori* strains, among which 62.6% were resistant to MTZ, 17.6% to AMX, 21.9% to CL, 19.8% to TC, and 16.7% to LE. Moreover, 19.5% of the strains have dual resistance to MTZ+LC. From Poland were included 1178 *H. pylori* strains, among which 48.5% were resistant to MTZ, 0% to AMX, 31.1% to CL, 7% to TC, and 8.1% to LE. Dual resistance to MTZ+LC was observed in 18.5% of the strains. The statistical difference has been demonstrated between both countries in resistance of *H. pylori* to MTZ ($p=0.0426$) and to AMX ($p<0.0001$). There were no statistically significant differences in the cases of resistance to CL, TC, LE, and dual resistance MTZ+CL. Summarizing, in Iran is the higher resistance of *H. pylori* strains to MTZ and AMX in comparison to Poland, in which so far strains resistant to AMX were not noted.

MICROBIOLOGICAL CONTAMINATION OF WATER IN FOUNTAINS LOCATED IN THE INOWROCŁAW HEALTH RESORT

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Fountains located in health resorts frequently serve as therapeutic waters intended for recreational and balneological purposes, therefore, its sanitary condition is important. An important problem is the simultaneous use of fountains by humans and animals, which can contribute to the transmission of pathogens and constitute an epidemiological threat. The aim of the study was to assess the degree of bacteriological and mycological contamination of water samples taken from fountains located in the Inowrocław health resort. Microbiological analysis of the water sample was taken from three fountains, one of which was supplied with brine water. Samples were collected 7 times in the period from June to October. The plate method was used to determination of a total count of mesophilic bacteria, Enterobacteriaceae bacteria and microfungi. For the determination of coliform bacteria, *Escherichia coli*, staphylococci and streptococci, the membrane filter technique was employed.

The studies showed that the samples of water from fountains contained bacteriological and mycological contamination in which microfungi being the most abundant group in the range from 33 to 176 CFU/1 ml. Of the indicator bacteria, the most frequent occurred staphylococci and their number was indicated at 22 to 68 cfu per 100 ml of water. In the analyzed water samples, there were only few cells of *Escherichia coli* and enterococci. It was indicated that the lowest number of microfungi and bacteria occurred in samples taken from a fountain with brine water. The presence of faecal bacteria, staphylococci, as well as microfungi in water may pose a risk of disease in people using fountains. Performed study indicates the need to conduct monitoring of water coming from fountains, as well as to introduce legal regulations regarding their sanitary and hygienic status.

Key words: Water, fountain, bacteria, microfungi, contamination.

ANTIMICROBIAL ACTIVITY OF LEAF EXTRACTS OF *PASSIFLORA* SPECIES ON SELECTED CLINICAL PATHOGENS

Marcin Ożarowski[1,2]*, Bogdan Kędzia[1], Małgorzata Kania-Dobrowolska[1], Justyna Baraniak[1], Aurelia Pietrowiak[1], Bogna Opala[1], Agnieszka Gryszczyńska[1], Elżbieta Hołderna-Kędzia[1]

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In recent years, researchers have shown increasing interest in the *Passiflora* genus due to its biological and pharmacological properties [1]. This species are an agronomically important crops and are used commercially in the fruit industry of South America [2]. Among of many ornamental plants, *P. alata*, *P. caerulea*, *P. citrina*, *P. incarnata* are most popular in the world. During of collection of fruits from cultivated plants, the leaves are removed. This plant material may be used for medicinal purposes. Our previous studies showed that crude extracts from leaves of *P. alata*, *P. caerulea* and *P. incarnata* contained various secondary metabolites such as phenolics, flavonoids, terpenoids [3]. Moreover extract of *P. alata* showed the most effective activities against *Acanthamoeba castellanii* strain *in vitro* [4]. The aim of our study was to evaluate and to compare the antibacterial and antifungal activities of the crude plant alcoholic extracts from leaf of *P. alata*, *P. caerulea*, *P. incarnata* and *P. citrina*. There was measurement of the minimal inhibitory concentration (MIC), the minimal bactericidal concentration (MBC), and the minimal fungicidal concentration (MFC) of the extracts.

Results showed that the most active extracts against *Enterococcus faecalis* (ATCC 8040) were as follows from: *P. incarnata* = *P. alata* (MIC=10.0 mg/ml, MBC>10.0 mg/ml) >*P. caerulea* (MIC=10.0 mg/ml, MBC>20.0 mg/ml); against *Escherichia coli* (PZH026B6): *P. incarnata* (MIC=10.0 mg/ml, MBC>10.0 mg/ml) >*P. caerulea* (MIC=10.0 mg/ml, MBC=20.0 mg/ml) >*P. alata* (MIC=10.0 mg/ml, MBC>20.0 mg/ml); against *Staphylococcus aureus* (ATCC 6538P): *P. incarnata* (MIC=2.5 mg/ml, MBC>5.0) [5] >*P. caerulea* (MIC=5.0 mg/ml, MBC>10.0) [5] >*P. citrina* (MIC=7.5 mg/ml, MBC>10.0) >*P. alata* (MIC=10.0 mg/ml, MBC>10.0) [5]; against *Candida albicans* (PCM1409PZH): *P. caerulea* (MIC=7.5 mg/ml, MBC=15.0 mg/ml), *P. incarnata* (MIC=10.0 mg/ml, MBC>10.0 mg/ml), *P. alata* (MIC=15.0 mg/ml, MBC>20.0 mg/ml); against *Microsporum gypseum* (K1): *P. incarnata* = *P. caerulea* = *P. alata* (MIC=5.0 mg/ml, MBC=5.0

mg/ml). Phytochemical study showed that the highest concentration of phenolic compounds was shown in extract of *P. alata*>*P. citrina*>*P. caerulea*>*P. incarnata*. There is a need for further studies of fractionated extracts and isolated compounds to estimate their activity.

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IN-VITRO ACTIVITY OF CEFTOLOZANE/TAZOBACTAM AGAINST CARBAPENEM-RESISTANT GRAM-NEGATIVE RODS RECOVERED FROM HOSPITALIZED PATIENTS OF A TEACHING HOSPITAL IN WARSAW, POLAND

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Ceftolozane/tazobactam is a novel antibiotic formulation composed of a cephalosporin - ceftolozane and beta-lactamase inhibitor - tazobactam. This formulation is approved for use in the treatment of complicated intra-abdominal and urinary tract infections caused by Gram-negative bacteria.

The goal of our study was to examine susceptibility of carbapenem-resistant metallo-beta-lactamase negative *Pseudomonas aeruginosa* and *Enterobacteriales* isolated from clinical specimens to ceftolozane/tazobactam.

The *in-vitro* effectiveness of the ceftolozane/tazobactam was tested for 49 strains, including *Enterobacter cloacae, Klebsiella pneumoniae* and *P. aeruginosa*. Minimum inhibitory concentration was determined using the E-test and interpreted according to the European Committee on Antimicrobial Susceptibility Testing guidelines.

Unlike *E. cloacae* and *K. pneumoniae* all the tested *P. aeruginosa* strains were susceptible to ceftozolane/tazobactam. The MIC value ranged from 2,0 to 6,0 μg/ml in case of *E. cloacae* and *K. pneumoniae* and from 0,5 to 4,0 μg/ml in case of *P. aeruginosa.*

The results confirmed the good *in vitro* activity of ceftozolane/tazobactam against carbapenem-resistant metallo-beta-lactamase negative *P. aeruginosa*.

BACTEREMIA CAUSED BY *CLOSTRIDIUM TERTIUM* - A CASE DESCRIPTION

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Clostridium tertium is a Gram-positive, endospore-forming, aerotolerant bacillus found in the soil and gastrointestinal tract of animals and humans. *C. tertium* was first isolated by Henry, in 1917 from the wounds of World Was I soldiers. To date, clinical significance of this bacterium is yet to elucidated, however an increasing number of report indicates this bacterium as a difficult to identify by standard procedures an emerging human pathogen.

Here, we describe the first case of bacteremia caused by *C. tertium* in a transplantological patient hospitalized in the Infant Jesus Teaching Hospital.

74-year old male patient, who underwent successful kidney transplantation in 2016, was admitted in 2018 to the hospital because of acute appendicitis. This condition was followed by fecal peritonitis and septic shock. Patient required hospitalization in the Intensive Care Unit. Gram stain morphology of bacteria from blood cultures indicated the presence of Gram-negative rods, unidentified by the use of biochemical methods and further identified by MALDI-TOF mass spectrometry as *C. tertium*. This patient responded well to the applied antimicrobial therapy.

In this case study we demonstrate how diagnosis and treatment can be challenging due to morphology and variable antibiotic resistance pattern.

MOLECULAR DIVERSITY AND EVOLUTION OF *BLA*$_{TEM}$ GENES ENCODING BETA-LACTAMASES IN *NEISSERIA GONORRHOEAE* STRAINS

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Introduction: The resistance to benzylpenicillin of *Neisseria gonorrhoeae* strains is often the result of beta-lactamase production. This enzyme is encoded by the *bla*$_{TEM}$ plasmid gene. Of the 223 known bacterial TEM beta-lactamases, five was detected in the *N. gonorrhoeae* e.g.: TEM-1 (the most common), TEM-135 and TEM-220. In other bacterial species (e.g. *Klebsiella pneumoniae, Escherichia coli*) beta-lactamase TEM may hydrolyzed additionally cephalosporins and monobactams.

Aim: The aim of this work was to detect and analyze mutations in the *bla*$_{TEM}$ gene, which results in resistance to penicillin of *N. gonorrhoeae* strains.

Material and methods: 333 strains of *Neisseria gonorrhoeae* obtained in 2013-2014 from the Department of Diagnostic Sexually Transmitted Diseases, Department of Dermatology and Venereology Medical University of Warsaw was examined. *N. gonorrhoeae* susceptibility to beta-lactam antibiotics (benzylopenicillin, ceftriaxone) was evaluated using the disc-diffusion method. Beta-lactamase was detected using cefinase test and *bla*$_{TEM}$ gene was detected by PCR. Analysis of mutations in *bla*$_{TEM}$ was carried out using sequencing method.

Results: Resistance to penicillin was observed in 49 (14.7%) strains of *N. gonorrhoeae*. 21 of strains (6.3%) produced the beta-lactamase. *bla*$_{TEM}$ gene was detected in all 21 strains using PCR method. Beta-lactamase TEM-135 was detected in 8 strains using sequencing method. This is the first report of presence of beta-lactamase TEM-135 of *N. gonorrhoeae* in Poland.

Conclusions:

1. Bla-TEM 135 gene was detected often in beta-lactamase-positive strains of *N. gonorrhoeae*
2. There may be locally clone *N. gonorrhoeae* encoding TEM-135.

TRANSFORMATION OF FLUOROQUINOLONE-RESISTANCE *NEISSERIA GONORRHOEAE GYR*A, *PAR*C GENES

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Mutations in the genes *gyr*A and *par*C coding the subunits enzymes topoisomerase II (gyrase) and topoisomerase IV are important mechanisms of resistance in fluoroquinolone-resistant bacteria, including *Neisseria gonorrhoeae*.

Strains were grown on chocolate agar plates at 37°C in 5% CO_2. Three donor strains were both highly resistant to ciprofloxacin and both had two *gyr*A and one *par*C mutation. Donor strains isolated from clinical material from the Department of Diagnostic Sexually Transmitted Diseases, Department of Dermatology and Venereology Medical University of Warsaw was examined. Two recipient clinical strains were a ciprofloxacin susceptible strains. Two more recipient strains were strain control strains: ATTC 19424 and ATTC49226. The MIC of ciprofloxacin was determined by E-test on chocolate agar. Extracted donors DNA was then added to the buffer TRIS, and incubated with strain recipient suspension for 4 h. Then, 150 µl of the cell suspension was cultured on each chocolate agar plate without and with selected concentrations of nalidixic acid 0.5 mg/L, 1 mg/L, 4 mg/L, 8 mg/L, 16 mg/L and 32 mg/L. PCR reactions was performed for the genes *gyr*A and *par*C, and the amplification products were sequenced and analyzed with sequences of donors and recipients.

N. gonorrhoeae has a formidable ability to develop resistance to various antibiotics, and perhaps most remarkable is the worldwide rapid increase in ciprofloxacin resistance during the last few years. The present study has shown that mutations in *gyr*A only caused a significant increase in the MIC to >32mg/L. The high resistance increase was due to three point mutations in the *gyr*A gene coding for the subunit of the gyrase. Mutations in the *par*C gene had no significant effect on ciprofloxacin resistance.

CHARACTERIZATION OF MICROORGANISMS ISOLATED FROM THE PHARMACEUTICAL ENVIRONMENT AND EVALUATION OF THEIR ABILITY OF BIOFILM FORMATION

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Cases of drug-related infections noted in the 1960s drew attention to the microbiological quality of drugs which are not required to be sterile. Investigations found that one of the root causes of microbial presence in pharmaceuticals was inadequate manufacturing process.

The aim of the study was twofold: to characterize microorganisms isolated from the manufacturing environment in pharmaceutical plants and to assess their biofilm-forming ability.

During the study, a total of 84 microbial strains were isolated from the facilities of three pharmaceutical plants, and identified. The most numerous group comprised bacteria from the genus *Pseudomonas*, the dominant species being *P. aeruginosa* (n=46). The highest proportion of bacteria (85.7%) was isolated in the vicinity of water fixtures (drains and sinks). A total of four bacterial species were isolated from production equipment including *Enterobacter cloace*, *Staphylococcus saprophyticus*, *Pseudomonas putida* and *Staphylococcus epidermidis*. The dominant microbes identified by swab tests on work tables were gram-positive cocci. All the identified bacteria were evaluated for ability to form biofilm. Strains showing a strong biofilm-forming ability represented 68% of all isolates. Six strains, including *S. saprophyticus*, *M. luteus*, *C. freundii* and *P. stutzeri*, were found not to produce any biofilm. The group of pathogens having a high biofilm-forming ability was dominated by *P. aeruginosa*. Qualitative identification of genes involved in the Quorum Sensing system and genes responsible for the secretion of virulence factors performed for *P. aeruginosa* rods isolated from the pharmaceutical production environment showed that 32 strains had all the genes participating in the QS. The gene responsible for the secretion of exotoxin A was present in 97.8% of all strains, and the gene responsible for elastase secretion was identified in 43 test strains. The gene responsible for the production of protease IV was found in the smallest number of isolates.

The results of the study demonstrate that the pharmaceutical environment is a source of potentially pathogenic microorganisms which may contaminate drugs manufactured on the premises and thus contribute to patient infections.

WHY ARE ANTIBIOTICS SOMETIMES NOT EFFECTIVE?

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Antibiotics are not effective because we abuse them and use them incorrectly. In patients with symptoms of infection, we usually use an antibiotic, and the cause of infection can also be viruses, fungi, parasites. Often, the antibiotic is not effective, because the pathogen is not in its spectrum. When we give the antibiotic empirically, there is a good chance of failure of therapy. When we give the targeted antibiotic drug may also be not active. Sometimes combination therapy is necessary. We need to predict what microbes are the cause of infection and use our knowledge about the spectrum of individual antibiotics. We need to remember about the natural and acquired resistance when we choose antibiotic. Adding comments to the result of a microbiological tests is a way to use the right antimicrobial drugs. Eradication of some microorganisms is associated with the generation of others. The phenomenon of bacterial resistance to antibiotics was already predicted by Fleming. An important problem is the lack of monitoring of the drug concentration. Today, for some multi-drug resistant strains, it is recommended to extend the therapy and use higher doses of antibiotics. The important phenomenon in antibiotic therapy are the “inoculum effect”, a biofilm, the interactions between medications and “clinical resistance” phenomenon. Because of a change in the etiological factor of the infection during antibiotics therapy, microbiological monitoring of the patient is necessary. The key to success is the individualization of therapy, sometimes off-label treatment, carrier screening tests and control over the use of antibiotics. Gut microbiome should be protected in the era of antibiotics resistance by diet, probiotics. The best way to reconstruct the gut microflora after prolonged and repeated antibiotic therapy isfaecalmicrobiota transplantation.

CALPROTECTIN LEVELS IN STOOL SAMPLES OF PATIENTS WITH *CLOSTRIDIUM DIFFICILE* INFECTION

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Clostridium difficile infection (CDI) can cause of nosocomial diarrhea and intestinal inflammation. Diagnosis of this condition is essential in the prognosis of severe CDI. One of these sensitive markers of intestinal inflammation is calprotectin. The aim of the study was to evaluate the level of calprotectin in patients with CDI and study of relationship between level of calprotectin and PCR-ribotype (RT) of *C. difficile*. The study involved two groups of samples: 58 stool samples from patients diagnosed with CDI, in which *C.difficile* toxins and glutamine dehydrogenase - GDH were found. Second group were 17 samples from healthy adults. All samples (75) were plated on CLO selective agar (bioMerieux, France) to grow a *C. difficile* strain. From the 58 diarrhea stool samples, 47 strains of *C. difficile* were cultured. RT was defined using specific primers. In none of the 17 stool samples from healthy people, the *C. difficile* strain was not cultured. C. difficile strains belonged to RT027 (n=39), RT020 (n=4), RT176 (n=2), RT001 (n=1), RT017 (n=1). The examination of calprotectin concentration in stool samples was performed with the EIA Ridascreen® Calprotectin test (R-Biopharm AG). The concentration of calprotectin <50 μg/g stool is considered to be ”normal”, in the range of 50-100 μg/g stool is slightly positive, concentration of calprotectin over 150 μg/g stool is considered to be ”elevated”. In 57 stool samples, concentration of calprotectin ranged from 151.5 μg/g to 4730.5 μg/g stool, which indicates on active inflammatory process (>150μg/g stool). In the 17 samples from the healthy people, the calprotectin level range were 44.5-47.0 μg/g stool, which is <50 μg/g stool and exclude intestinal inflammation. In the one stool sample calprotectin level was 96.3 μg/g stool which mean weak-positive result. From the tested samples, concentration of calprotectin in stool of patients infected by strains belonging to RT027 ranged from 96.3 μg/g to 4730.5 μg/g, RT176 from 209.7 μg/g to 4394.4 μg/g, RT020 from 361.2 μg/g to 3454.5 μg/g, RT 001, the concentration was 3525.8 μg/g, and RT017 concentration was 2230.5 μg/g. The study found a differentiated level of calprotectin in the gastrointestinal tract in patients with CDI. There were no statistically significant differences in the concentration of calprotectin between patients infected with different PCR-ribotypes. However, it should be emphasized that these tests carried out on a small number of samples. Examination of relationship between level of calprotectin and genetic type of *C. difficile* causing CDI requires further investigation.

INFLUENCE OF SELECTED CONCENTRATIONS OF TARRAGON ESSENTIAL OIL ON FATTY ACID PROFILE IN CELLULAR MEMBRANES OF *PSEUDOMONAS* SPP.

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There is an increasing interest in essential oils as an alternative means of prolonging food durability. Essential oils occur naturally in nature, do not cause side effects and have antimicrobial activity even at low concentrations. Antimicrobial activity is closely related to the composition of essential oil and is usually the result of a complex and multidirectional cascade of reactions. Under the influence of essential oils there are, among others, changes in the profile of fatty acids present in cellular membranes. This phenomenon leads to disturbances in the permeability of the membrane. The above modification of the structure of cellular membranes also leads to changes in the enzymatic activity and excessive outflow of cellular components. These changes ultimately lead to inhibition of the metabolic activity of microbial cells.

The aim of the study was to evaluate the effect of selected concentrations of essential oil from tarragon on the fatty acids profile in cellular membranes of selected *Pseudomonas* spp.

The strains belonging to the species *Pseudomonas fluorescens*, *Pseudomonas psychrophila* and *Pseudomonas orientalis* were subjected to investigations. All microorganisms were isolated from spoiled fish products and identified by analysis of the 16S rRNA sequence. The essential oil of tarragon (*Artemisia dracunculus* L.) was obtained in the process of hydrodistilation in the Derynga apparatus. Identification of the components of the tarragon essential oil was carried out using gas chromatography coupled with mass spectrometry (GC-MS). Determination of sub-inhibition concentrations of essential oil was carried out using the traditional method of the broth macrodilution and flow cytometry technique. The tested microorganisms were incubated on TSB medium supplemented with selected concentrations of the essential oil. Incubation process was carried out at 15 °C for 72 hours. After this time, fatty acids were extracted and methylated from biomass. Methyl esters of fatty acids in the samples were identified by gas chromatography (GC).

Simultaneous application of the broth macrodilution method and flow cytometry technique allowed for proper specification of the sub-inhibitory concentrations of tarragon essential oil against the tested *Pseudomonas* spp. strains. The presented work has differentiated the influence of

tarragon essential oil on the fatty acid profile extracted from cellular membranes of *Pseudomonas* spp. In *P. fluorescens* the decrease concentration of saturated 14C fatty acids and increase concentration of saturated 18C fatty acids were observed. In *P. psychrophila* and *P. orientalis* strains decrease content of unsaturated 16C and 18C fatty acids were observed. The essential oil of tarragon showed an efficient antibacterial effect even at sub-inhibitory doses and can be successfully used to preserve foods and other products.

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ANTIMICROBIAL PROPERTIES OF ESSENTIAL OILS OF SELECTED CULINARY HERBS AND SPICES

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Due to an increasing drug resistance of some dangerous strains of microorganisms, academic medicine is looking for new ways to treat many infectious diseases. In the future, a good alternative to synthetic drugs used in medicine may be essential oils which have a wide range of biological and pharmacological activity. Fundamental for a use of preparations based on essential oils as natural antimicrobial remedies is finding out the relationship between the chemical composition of oils and their antimicrobial effect. Contemporary scientific research confirms antibacterial, anti-inflammatory and antiseptic activity of many aromatic plants used in food preparation. These properties concerning common and widely available raw materials can be used in prevention, control and treatment of bacterial infections in humans and animals. The chemical composition and antibacterial activity of essential oils from 6 commonly consumed spice plants: clove *Syzygium aromaticum* (L.) Merr. & Perry, all spice *Pimenta dioica* (L.) Merr. (Myrtaceae), cinnamon *Cinnamomum verum* J. S. Presl (Lauraceae), thyme *Thymus vulgaris* L., oregano *Origanum vulgare* L., and rosemary *Rosmarinus officinalis* L. (Lamiaceae) have been analyzed in scientific literature.

The highest bactericidal and bacteriostatic action and the widest spectrum of activity has been documented for *T. vulgaris, O. vulgare* and *S. aromaticum*. The analysis of the research shows that among the phenolic components of essential oils, the highest antibacterial activity was demonstrated by cinnamaldehyde, thymol, carvacrol and eugenol, while among terpenic compounds by borneol and camphor. Furthermore, the effect of essential oils and their components is sometimes stronger than antibiotics. It has been proved that among the components of oils, thymol and carvacrol had the strongest properties, even superior to streptomycin. Thyme oil has been shown to be an effective antibacterial agent against drug-resistant strains. The study of thyme oil, thymol and carvacrol confirmed that essential oils often have a stronger antibacterial effect than their individual substances which indicates synergy in action of oils compounds.

DIETARY SUPPLEMENTS: PLANT COMPONENTS WITH ANTIMICROBIAL AND IMMUNE SUPPORTING PROPERTIES

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On the Polish pharmacy market, numerous dietary supplements with plant extracts are available. Preparations containing ingredients with documented antibacterial, antiviral and antifungal properties and supporting the functioning of the immune system belong to the important group of these products. They are recommended in the autumn-winter and spring solstice periods, prophylactically in states of the weakness of the body and reduced immunity or additionally during infections of the upper respiratory tract (colds, runny nose, inflammations of the throat, etc.).

Based on the preliminary research, a list of over 70 above described products was prepared. These dietary supplements have a different form (capsules, tablets, syrups, etc.), varied composition, and plant ingredients occur individually or a few (usually 4-5 herbs). Among the plant active compounds, the extract of acerola *Malpighia glabra* L. (primarily as a rich source of vitamin C), extract or juice from elder fruits *Sambucus nigra* L. (with a high content of anthocyanins and other polyphenols), and an odorless extract from garlic *Allium sativum* L. (usually in the combination with vitamin D_3 and/or shark liver oil) were most often recorded. As the typical products, we found several-component preparations with linden flower and elder flower/fruit in the combination with plant raw materials protecting the upper respiratory tract (e.g. root of African geranium *Pelargonium sidoides* DC. and marshmallow *Althaea officinalis* L.). Attention was also paid by quite numerous dietary supplements containing extracts from plants strengthening the vital forces of the body (mainly ginseng root *Panax ginseng* C.A. Meyer, less turmeric rhizome *Curcuma longa* L.) and species with immunostimulatory properties (herb and root of purple coneflower *Echinacea purpurea* (L.) Moench, herb of hairy rockrose *Cistus incanus* L., and aloe leaf *Aloe barbadensis* Mill.).

The above-mentioned products constitute a concentrated source of vitamins (mainly vitamins C and D_3 as well as A, E, and vitamins of group B), minerals (primarily Zn and Se) and plant bioactive compounds. Therefore, they can be used as preparations supporting the immune system: prophylactically and during infection. However, as food products, they should not replace drugs, including herbal medicines.

QUALITATIVE AND QUANTITATIVE EVALUATION OF HSV-1 VIRUS IN SAMPLES OBTAINED FROM PATIENTS WITH HERPETIC KERATITIS USING PCR AND REAL-TIME PCR

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Herpes simplex keratitis, caused mostly by HSV-1 (*Herpes Simplex Type 1*) is known as herpetic keratitis. It is the main cause of blindness and visual morbidity in developing countries. Recurrent infectious or immune keratitis cause structural damage to the cornea, scarring, and may lead to blindness. Thus, there is a need to find the simple and non-invasive method to identify this virus presence and prevent the new herpes cases.

The aim of the study was the qualitative (using *PCR*) and quantitative (using *real-time PCR - qPCR*) identification of HSV-1 virus in swabs obtained from the eyes surfaces of patients with diagnosed herpetic keratitis.

Material was collected using FLOQSwabs™ Flocked Swabs (Copan Diagnostics, USA) and eNAT™ preservation/lytic medium (Copan Diagnostics). Viral DNA was extracted using Syngen Viral Mini Kit (Syngen Biotech®, Poland). Qualitative virus identification was carried out using PCR reaction with specific primers sequence for *thymidine kinase* gene (*UL23).* qPCR reactions were performed using Fast Start Essential DNA Green Master (Roche Diagnostic, Switzerland). The reactions were carried out using LightCycler instrument (Roche, Switzerland).

Discrepancies in identification results between qualitative and quantitative approaches were found. In contrast to qualitative reactions, real-time PCR results for HSV-1 for all samples were positive. The amount of the viral DNA was in the mean level from 7.5×10^0 to 8.0×10^1 DNA copies per 1 µl.

Genetic techniques may be alternative to classical methods for the identification of microorganisms such as HSV-1.

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ANTIFUNGAL ACTIVITY OF QUATERNARY AMMONIUM SALTS (QAS) AGAINST *CANDIDA ALBICANS*

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Quaternary ammonium salts (QAS) are cationic surfactants with antimicrobial activity. These compounds are widely used in medicine, industry and agriculture (as disinfectants, drugs, biocides and fungicides).

Two groups of cationic surfactants were compared: monomeric QAS (DMGM-16: 2-hexadecyloxy-N,N,N-trimethyl-2-oxoethanammonium bromide) and dicephalic one (C_{16}(TAPABr)$_2$: N,N-bis[3,3'- (trimethylammonio)propyl]alkylamide dibromide). The antifungal activity of DMGM-16 and C_{16}(TAPABr)$_2$ against the pathogenic strain *Candida albicans* ATCC 10231 was studied. The both have been shown to inhibit the growth of *C. albicans* and the process of filamentation in this strain as well. Moreover, the anti-adhesive effect on the polystyrene surface was checked but only dicephalic salt C_{16}(TAPABr)$_2$ was effective in that field. A 16-carbon cationic surfactant DMGM-16 effectively eradicated the biofilm produced by *C. albicans* on the polystyrene surface and both salts influenced the viability of the cells in the biofilm structure.

AN INFLUENCE OF SELECTED ESSENTIAL OILS ON BACTERIA *PSEUDOMONAS ORIENTALIS*

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The growing aversion of consumers to synthetic preservatives leads to the search for new substances with similar functions. Essential oils are mixtures of aromatic compounds, found in the cells of oil plants. These substances are increasingly considered as natural food preservatives due to the confirmed strong antimicrobial activity. The essential oils of lime, lemongrass, juniper, black pepper and rosemary have an inhibitory activity on the growth of *Pseudomonas orientalis*, which are responsible for the spoiling of animal products, especially fish. The strongest action was demonstrated by the oils of lemon grass and black pepper. The strongest activity was demonstrated by the oils of lemongrass and black pepper. Research has confirmed the great importance of synergy between the components of oils in their general activity. The studied essential oils caused the rounding od *Pseudomonas orientalis* cells. The addition of oils causes a significant decrease in the number of bacteria immediately after the addition, and then maintaining the number of cells at a constant level. The tests have confirmed the possibility of using essential oils in the form of preservatives. The addition of lemon and lemongrass oils in MIC concentrations to the raw salmon filet resulted in a reduction in the number of *Pseudomonas orientalis* bacteria, increasing the storage stability of this product. Baked salmon filet with the addition of lemon and lemongrass oils was characterized by very good sensory qualities.

QUORUM QUENCHING – A STRATEGY AGAINST BIOFILM FORMATION AND FOOD SPOILAGE REACTIONS OF *PSEUDOMONAS* SPP.

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A *quorum sensing* system plays an important role in the process of adhesion of microorganisms to abiotic surfaces or food spoilage, therefore identifying ways to interfere with this mechanism may prove important information to the food industry. It has been proven that many natural compounds are capable of inhibiting *quorum sensing*. The above antimicrobial activity may be manifested by: (i) reducing the autoinductor synthase activity; (ii) stop the production of autoinducers or reduce the efficiency of this process; (iii) degradation of autoinductor molecules; (iv) replacement of autoinducers produced by microorganisms with synthetic substances of similar chemical structure. Among the various possibilities of interfering with the *quorum sensing* system, inhibition of the synthesis of autoinductor molecules is considered to be the most promising strategy for the effective elimination of undesirable microorganisms from food and the production environment.

Each of the compounds used to inhibit autoinductors synthesis should have the following characteristics: (i) they should be low molecular weight substances that selectively affect the degree of gene expression; (ii) they should be substances that exhibit specificity for *quorum sensing* regulators, and (iii) they should be chemically stable in the food matrix or in the formulated preparations. Potential inhibitors of the *quorum sensing* mechanism of *Pseudomonas* spp. should also not exhibit cytotoxic and genotoxic effects on human gastrointestinal cells. The above criteria for *quorum sensing* inhibitors may be fulfilled by phenolic acids and other bioactive compounds present in plant essential oils, but detailed studies in this field have not yet been undertaken. Consequently, in the work, a real assessment of the potential of selected phenolic acids and essential oils from spice to interfere with the bacterial *quorum sensing* were investigated.

In the work the following aspects are considered to be the most scientifically valuable:

- use in all experiments native strains, isolated from food and production environment. In addition, application of experimental systems that simulates industrial conditions or in model food products;
- conducting interdisciplinary research, confirming that the *quorum sensing* mechanism of microorganisms plays an important role in the specificity of microbiological spoilage of food. It also determines the rate of colonization of utility surfaces by microorganisms;

- proposing new analytical protocols, which are a compilation of conventional and alternative methods and modern ones. This enabled to precisely determine the sub-MIC concentrations of antimicrobial agents tested, and to study the dynamics of bacterial AHL autoinductors synthesis;
- demonstration that bioactive compounds of natural origin, by affecting the synthesis of bacterial *quorum sensing* autoinductors, simultaneously cause suppression of target genes. They can in this way delay the process of spoilage of materials. This discovery can be applied in the design of modern preparations for preserving foods or preparations for abiotic surfaces.

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BIOMEDICAL APPLICATION OF BACTERIOCINS

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Introduction: Bacteriocins are small peptides with antimicrobial activity. Four classes of bacteriocins have been defined on the basis of structural characteristics. Bacteriocins that are produced by lactic acid bacteria are thought to be eaten since ancient times with many of foods (dairy products and fermented vegetables). Nowadays they are useful as food preservatives but they also could be used for medicinal purposes e.g. dealing with antibiotic-resistant bacteria. Moreover they are reported as therapeutic agents (e.g. microbiota regulators), alternative antiviral substances and even anticancer agents.

Divercin AS7, which is the subject of the studies, is a class IIa bacteriocin produced by *Carnobacterium divergens* AS7 and is particularly effective against human pathogen *L. monocytogenes.* This bacteriocin could be used beyond food and feed safety and quality. Here are presented results of studies on divercin AS7 properties (biosafety) and also summarized current knowledge about biomedical application of bacteriocins produced by lactic acid bacteria.

Materials and Methods: Heterologous peptide production is way to achieve synthesis of bacteriocin to support biochemical and biomedical studies. In order to study expression of divercin AS7 in different heterologous hosts, its mature native coding sequence was successfully cloned in series of vectors allowing its synthesis in *E.coli* BL21DE3pLys, *P. methanolica* pMAD11 and pMAD16 and *L. lactis* MG1363 strains. To analyze impact of divercin AS7 on human enterocytes *in vitro* studies were conducted (cell integrity, cytotoxic effect and apoptosis). The yeast two-hybrid system (*S. cerevisiae* MaV203) was used to identify *in vivo* interactions between divercinAS7 and human proteins.

Results: Divercin AS7 is effectively synthesized in heterologous hosts *E. coli*, *P. methanolica* and *L. lactis*. Its recombinant versions retain antibacterial activity and thermal stability. The absence of an apoptotic effect, lacks of cytotoxicity, no damage to enterocytes allow to conclude that recombinant divercin AS7 is not toxic to human cells. Interaction, crucial for molecular processes such as translation, intracellular trafficking and oncogenesis, of divercin AS7 with human epithelial proteins were identified.

IMPACT OF THE GUT MICROBIOME ON FLAVONOID METABOLISM

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Dietary flavonoids represent a diverse range of polyphenolic compounds that are present in many commonly consumed fruits, vegetables, grains, herbs, and beverages. The structural complexity of flavonoids has led to their subclassification as flavonols, flavones, flavan-3-ol, isoflavones and anthocyanins. The diversity of flavonoid structures undoubtedly contributes to differences in biological efficacy with subtle differences affecting both bioavailability and bioactivity. A number of factors, including age, sex, and genotype, may affect these metabolic processes. Flavonoids are extensively metabolized by phase I and phase II metabolism (which occur predominantly in the gastrointenstinal tract and liver) and colonic microbial metabolism. Colonic metabolism has long been speculated to be a major contributor to the overall metabolism of not only dietary flavonoids but also of phase I and II metabolites that have been excreted back into the intestine via enterohepatic circulation. The bacterial enzymes deglycosylate the compounds, but the microbes can also perform a range of other transformations including oxidation, demethylation, and the catabolism to smaller fragments including small phenolic acids and aromatic catabolites. However, it remains unclear how well these metabolites are absorbed. The colonic bioconversion of flavonoids is thought to be highly variable although the etiology of the heterogeneity is currently unclear.

Keywords: microbiome, flavonoids, health, metabolism.

THE ROLE OF HPV IN OVARIAN CANCER

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Ovarian cancer is the fifth leading cause of female cancer deaths. More than 75% of women who present locally advanced or disseminated disease are characterized by a gradual invasion of the surrounding organs and, in high-stage cases, invasion of the peritoneal cavity. The survival rate of patients presenting with widespread metastatic disease is only approximately 20%. More than 100 types of human papillomavirus (HPV) are known, and they are categorized into 3 broad categories depending on their oncogenic potential. A link between HPV infection and squamous cell cancer of the cervix has been identified. The carcinogenic potential of high-risk HPV is well described and includes several mechanisms. Viral oncogenes E6 and E7 are pivotal elements, since expression of these genes impairs the function of host-cell tumor suppressor p53 and retinoblastoma protein, thus favoring malignant transformation. Furthermore, molecular studies have demonstrated the capability of E6 and E7 to target cellular factors like paxillin, tuberin, E6-AP, E6-BP, E6TP1 and TNFR-1 as well as cell cycle regulators such as cyclins, cyclin-dependent kinases and CDKs-inhibitors.

Keywords: Ovarian cancer, HPV.

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