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3RD INTERNATIONAL CONFERENCE

ANTIMICROBIAL RESISTANCE - CURRENT STATE AND PERSPECTIVES

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PROGRAM 17th of May, 2024.

17th of May, 2024.		
08:00-09:00	Registration of the participants	
09:00-11:00	**Session 1. Current state of antimicrobial resistance** Moderators: ***Milica Bajčetić,*** full professor Department of Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Belgrade, Serbia ***Ahmed Smajlović,*** full professor Veterinary Faculty, University of Sarajevo, Bosnia and Herzegovina	
	Can alternatives to antibiotics replace the use of antibiotics in livestock production? ***Luca Guardabassi***, full professor Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences University of Copenhagen ***Denmark***	
	Antimicrobial resistance surveillance in Serbia – Data from human national reference laboratory ***Deana Medić***, associate professor Faculty of Medicine, University of Novi Sad, Institute of Public Health Vojvodina ***Serbia***	
	Antimicrobial resistance surveillance in Serbia – Data from veterinary national reference laboratory ***Dalibor Todorović***, research associate Scientific Veterinary Institute 'Novi Sad' ***Serbia***	
	Recent trends in antimicrobial resistance of bacteria from livestock and meat in the Netherlands – from a One Health perspective ***Kees Veldman***, senior scientist National Reference Laboratory on antimicrobial resistance in animals, Wageningen Bioveterinary Research ***Netherlands***	
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	One health approach in AMR and antibiotic consumption control in Serbia – five years of the national multi-sectoral coordinating group activities ***Milica Bajčetić,*** full professor Department of Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Belgrade ***Serbia***	**Influence of cross-contamination of animal feed with antimicrobials on intestinal microbiome** ***Małgorzata Olejnik***, associate professor Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Torun ***Poland***
	The economic cost of antimicrobial resistance: FAO Perspective ***Diletta Topazio***, economist Food and Agricultural Organization of the United Nations; teaching assistant, LUISS Guido Carli University, Rome ***Italy***	**Association of AMR in E. coli from calves raised for meat production with husbandry system** ***Tenhagen Bernd-Alois***, senior researcher Federal Institute for Risk Assessment, Berlin ***Germany***

PROGRAM 17th of May, 2024.

	Different aspects of Hungarian antibiotic use *Ria Benkő*, associate professor Faculty of Pharmacy, University of Szeged *Hungary*	**Evaluation of antimicrobial resistance of *Pseudomonas* spp. isolates from food** *Radoslava Savić Radovanović*, associate professor Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, University of Belgrade *Serbia*
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18th of May, 2024.		
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	Biosecurity in animal production to improve animal health and reduce antimicrobial use *Jeroen Dewulf*, full professor Department of Internal medicine, Reproduction and population medicine, Faculty of Veterinary Medicine, University of Ghent ***Belgium***	**Antimicrobial resistance surveillance in Republic of North Macedonia with focus on carbapenem resistant Enterobacteria as an actual problem in clinical practice** *Ana Kaftandzieva*, full professor Institute of Microbiology and Parasitology, Ss. Cyril and Methodius University in Skopje ***Republic of North Macedonia***
	Biosecurity in poultry production in Italy *Alessandra Piccirillo*, associate professor Department of Comparative Biomedicine and Food Science, University of Padua ***Italy***	**Lactonase mediated quorum quenching of *Pseudomonas aeruginosa* virulence** *Milka Malešević*, research associate Institute of Molecular Genetics and Genetic Engineering, University of Belgrade ***Serbia***
	Current status of biosecurity measures in the pig production sector in Serbia *Jasna Prodanov Radulovic*, senior research associate Scientific Veterinary Institute 'Novi Sad' ***Serbia***	**The impact of antibiotic interactions on the pathogens generating resistant hospital infections** *Bojana Bozic Cvijan*, assistant professor Department of Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Belgrade ***Serbia***
	Antimicrobial therapy: advances and challenges facing veterinary practitioners in Bulgaria *Aneliya Milanova*, full professor Department of Pharmacology, Animal Physiology, Biochemistry and Chemistry, Faculty of Veterinary Medicine, Trakia University ***Bulgaria***	**The role of the clinical pharmacologist in rationalizing the use of antibiotics - experiences from the Osijek Clinical Hospital Center, Croatia** *Suzana Mimica*, associate professor Department of Pharmacology, Faculty of Medicine University of Osijek, Department of Clinical Pharmacology, University Hospital Centre Osijek ***Croatia***
	Prudent antimicrobial use on the poultry farms in Serbia *Marko Pajić*, research associate Scientific Veterinary Institute 'Novi Sad' ***Serbia***	**Assessment of seasonal airborne resistome dynamics in response to air pollution exposure in the Belgrade metropolitan area** *Katarina Novović*, senior research associate Institute of Molecular Genetics and Genetic Engineering, University of Belgrade ***Serbia***
	Metabolic gene expression signatures in response to residual doses of antibiotics in broiler chickens ***Anna Sławińska***, associate professor Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Torun ***Poland***	**Current status of multidrug resistant organisms in critically ill children-a growing concern: Report from the Institute for child and youth health care in Vojvodina, Serbia** *Isabela Fabri*, assistant professor, Institute for Child and Youth Health Care Vojvodina, Faculty of Medicine, University of Novi Sad ***Serbia***
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	Presentation of general sponsor - Topigs Norsvin Correlation between antibiotic use and pig genetics ***Laszlo Buza***, associate professor University Veterinary Medicine Budapest ***Hungary***	

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	Presentation of bronse sponsor – Sigma Aqualor H disinfection products - proven effectiveness in the prevention of African swine fever ***Bojan Toholj,*** full professor Departmant of Veterinary Medicine, Faculty of Agriculture, University of Novi Sad ***Serbia***	
12:30-13:00	Coffee break	
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	Do we need new drugs in therapy of Otitis externa, or do the old ones still work? ***Natalija Milčić Matić,*** teaching assistant ***Isidora Prošić,*** teaching assistant Faculty of Veterinary Medicine, University of Belgrade ***Serbia***	**Antifungal-resistance from both human health and plant protection perspectives** ***Li-Jun Ma,*** associate professor College of Natural Sciences, University of Massachusetts ***United States of America*** **Environmental impact of antibiotic use in plant protection** ***Milan Ivanović,*** full professor Faculty of Agriculture, University of Belgrade ***Serbia***
	Phage therapy of skin dysbiosis in dogs ***Tina Kotnik,*** associate professor Veterinary Faculty, University of Ljubljana ***Slovenia***	**Formation of soil resistome** ***Simonida S. Đurić,*** full professor Faculty of Agriculture, University of Novi Sad ***Serbia***
	Effect of antimicrobials and probiotics on gut microbiota in companion animals ***Nataša Golić,*** full research professor Institute of Molecular Genetics and Genetic Engineering, University of Belgrade ***Serbia***	**Biosecurity issue in the common carp production** ***Miloš Pelić,*** research associate Scientific Veterinary Institute 'Novi Sad' ***Serbia***
	Antibiotic sensitivity of bacterial isolates from canine ejaculates ***Jelena Apić,*** senior research associate Scientific Veterinary Institute 'Novi Sad' ***Serbia***	**Wastewater from slaughterhouses: A risk for antimicrobial resistance dissemination throughout the One Health concept** ***Katerina Dodovska Blagoevska,*** senior research associate Faculty of Veterinary Medicine – Skopje, Ss. Cyril and Methodius University in Skopje ***Republic of North Macedonia***
	Human approved antimicrobials in the small animal practice: when and why? ***Zorana Kovačević,*** associate professor Departmant of Veterinary Medicine, Faculty of Agriculture, University of Novi Sad ***Serbia***	**Toward sustainable control of gray mold on strawberries:** **Exploring alternatives to synthetic fungicides considering resistance evolution** ***Marta Loc,*** teaching assistant Faculty of Agriculture, University of Novi Sad ***Serbia***
14:30-15:30	Lunch	
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Dear colleagues,

3rd International Conference "Antimicrobial resistance – current state and perspective" is event dedicated to antimicrobial resistance under the One Health umbrella, providing a unique collaborative environment between researchers, veterinarians, doctors, pharmacists and stakeholders. One Health approach will enable multicestoral collaboration between humans, animals and environment in solving problem of antimicrobial resistance as one of the major heath treaths.

The risk for the spread of bacterial resistance when antimicrobials are used is well known, and the interconnection of use of animal and human antimicrobials further increases the concern for human and animal health. Misusage and overuse of antimicrobials is one of the main drivers of antimicrobial resistnce, so increase attention and measures to promote an overall reduced and responsible use are necesery. Antimicrobials are still critically important and necessary in the treatment of animals and humans and we have to save their effectivnes for the future generations.

dr Zorana Kovačević, associate professor
president of the scientific and program committee

CONTENT

CAN ALTERNATIVES TO ANTIBIOTICS REPLACE THE USE OF ANTIBIOTICS IN LIVESTOCK PRODUCTION?

Luca Guardabassi[1*]

[1]Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark

*lg@sund.ku.dk

Antibiotics are widely regarded as a primary contributor to the selection and dissemination of antibiotic resistance in animals. Various alternative strategies are currently tested to minimize antibiotic usage, including gut microbiota modulators, dietary interventions, immunostimulants, and phages. These strategiesare being extensively explored in a current EU project called AVANT, comprising 14 academic and industrial partners across nine countries. The project specifically targets post-weaning diarrhoea in piglets, a major reason for antibiotic use in livestock production at the global level. Two interventions were chosen for clinical trials: an on-farm faecal filtrate transplantation (FFT) protocol and a non-fermentable fibre-based diet. Based on the results of pre-clinical studies, both interventions appear to decrease the frequency of post-weaning diarrhoea, therefore reducing the need for antibiotic treatment. However, they primarily focus on prevention rather than cure, and notably, they do not eliminate the disease. Further research is needed to enhance the efficacy and streamline the integration of alternatives to antibiotics in intensive animal farming. For instance, the protective effects of FFT could be enhanced by optimizing dosage, timing, and duration of administration. Economically viable methods should also be developed for selecting donors and producing and administering faecal filtrates on farms in a cost-effective manner. Moreover, the development of a regulatory framework is essential to allow testing and implementation of this practice in the field, especially considering the current EU legislation prohibiting the administration of any type of faecal derivatives to animals destined for food consumption. The need for legal regulation extends to other alternative strategies to reduce antibiotic use, such as phage therapy. It's important to acknowledge that, given the current state-of-the-art, fully replacing antibiotics with these alternatives in intensive livestock production systems is unlikely. The primary conditions requiring antibiotic use, such as post-weaning diarrhoea or bovine respiratory disease in cattle, are strongly associated with the stressful conditions inherent in the current animal production practices. Thus, achieving the societal goal of eliminating antibiotic use in intensive livestock production may require re-thinking of the methods used for raising animals. Antibiotic-free production offers a potential solution, where animals are raised in conventional farms under reduced stress conditions. If an individual animal falls ill, it receives treatment and is eventually slaughtered and sold conventionally. Success in antibiotic-free production entails a comprehensive approach, including stress reduction, immune-supportive diets, and stringent hygiene and disease prevention practices. In this context, alternatives to antibiotics could play a pivotal role in maximizing the future production of antibiotic-free animals by reducing disease prevalence and reliance on antibiotics in this emerging production system.

Key words: alternatives to antibiotics, antibiotic use, antibiotic resistance, faecal microbiota transplantation, high-fibre diets, synbiotics

Acknowledgments: The experimental work described during this lecture was supported by the Innovation Action “Alternatives to Veterinary Antimicrobials” (AVANT) funded by the European Union’s Horizon 2020 Research and Innovation Programme under Grant Agreement No 862829.

ANTIMICROBIAL RESISTANCE SURVEILLANCE IN SERBIA - DATA FROM HUMAN NATIONAL REFERENCE LABORATORY

Deana Medić[1,2*]

[1]Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia
[2]Institute of Public Health of Vojvodina, Center for Microbiology, Novi Sad, Serbia

*deana.medic@gmail.com; deana.medic@mf.uns.ac.rs

Antimicrobial resistance (AMR) is a global medical problem, resulting in the increased attention of not only the experts, but of the general public, too. It arises and spreads primarily because of the selective pressure of antibiotics, due to their excessive use and abuse in human medicine, as well as in veterinary medicine, agriculture and food production. Infections caused by multiresistant bacteria complicate treatment of patients, prolong patient stay in stationary health institutions, increase morbidity, mortality and costs. In 2008, the Ministry of Health appointed the National Reference Laboratory for Antimicrobial Resistance located in the Center for Microbiology of the Institute for Public Health of Vojvodina. Surveillance of antimicrobial resistance in Serbia is carried out in the National Reference Laboratory for AMR and through participation in the CAESAR (Central Asian and European Surveillance of Antimicrobial Resistance) network. The two main goals of this network are to collect comparable and valid AMR data and to analyse AMR prevalence and trends over time. Our country has been a member of this network since 2013. The pathogens monitored are *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Salmonella* spp., *Staphylococcus aureus*, *Enterococcus faecalis/faecium*, *Streptococcus pneumoniae*. Clinical antimicrobial susceptibility data for these bacterial specieswere collected from 24 clinical laboratories included in the National AMR surveillance network, with an estimated total national population coverageof 78%. All laboratories included in the National AMR surveillance network used EUCAST guidelines for antimicrobial susceptibility testing. In 2022, 3.431 primary isolates of invasive bacteria (from blood cultures and cerebrospinal fluid) were confirmed. The data show that a high level of resistance to 3rd generation cephalosporins, carbapenems, but also to colistin was recorded in *Klebsiella pneumoniae* - 91.7%, 65.7% and 35.4%, respectively. Although resistance to carbapenems in *Escherichia coli* was low - 1.5%, it was a cause for concern. *Pseudomonas aeruginosa* and *Acinetobacter* spp. show resistance to carbapenems - 49.4% and 97.5% respectively, while the resistance to colistin in these two microorganisms was low, at the rates of 1.3% and 1.9%, respectively. Resistance in Gram-positive bacteria were the following: methicillin resistance in *Staphylococcus aureus* – 28.6%, vancomycin resistance in *Enterococcus faecium* – 53.1% and penicillin resistance in *Streptococcus pneumoniae* – 40.5%. AMR is becoming a serious problem in Serbia. Serbia is still among European countries with the highest percentage of resistant isolates. According to the second joint report of the EARS-Net/CAESAR network, the rates of AMR in Serbia still correspond to the data of the countries of Southern and Eastern Europe.

Key words: invasive isolates, antibiotics, AMR

ANTIMICROBIAL RESISTANCE SURVEILLANCE IN SERBIA – DATA FROM VETERINARY NATIONAL REFERENCE LABORATORY

Dalibor Todorović[1*], Biljana Đurđević[1], Marko Pajić[1], Bojana Prunić[1], Igor Stojanov[1]

[1]Scientific Veterinary Institute „Novi Sad", Novi Sad, Serbia

*dalibor@niv.ns.ac.rs

The susceptibility testing of indicator and pathogenic bacteria to antibiotics is carried out in the National Reference Laboratory for Antimicrobial Resistance (NRL-AMR) in veterinary medicine using the disc diffusion method according to the European Committee for Antimicrobial Susceptibility Testing (EUCAST) and the microdilution method according to Directive 2020/1729 of the European Union. The genomic characterization of the bacteria is performed by polymerase chain reaction (PCR) and whole genome sequencing (WGS). The results of the NRL-AMR over the last five years are of great importance to One Health. ESBL/AmpC-producing *E. coli* resistant to fluoroquinolones (ciprofloxacin), aminoglycosides (gentamicin, neomycin and amikacin), polymyxins (colistin) and glycylcyclines (tigecycline) have been detected on pig and cattle farms and ESBL/AmpC-producing *E. coli* resistant to ciprofloxacin and gentamicin have been detected on poultry farms.Alarming is the resistance to nalidixic acid in *Salmonella* Enteritidis and the plasmid-mediated resistance in *S*. Infantis, *S*. Enteritidis and *S*. Kentucky serotypes from poultry farms. In addition, multidrug resistance was detected in the *S*. Infantis serotype and high resistance to ciprofloxacin was detected in farms with broiler chickens. Resistance of *E. coli* to ciprofloxacin was detected in wild animals (wild boar, deer, roe deer and rabbits) and wild birds (gulls and white-tailed eagles). Methicillin-resistant *Staphylococcus pseudintermedius*, which is resistant to fluoroquinolones, aminoglycosides, macrolides, sulfonamides, tetracyclines and phenicols, has been detected in healthy dogs in animal shelters. The monitoring of bacterial sensitivity to antibiotics in veterinary medicine in the Republic of Serbia has not yet been implemented. The results of the NRL-AMR draw the attention of the scientific and professional public to the need to conduct surveillance of antibiotic resistance in order to better understand the risks of the spread of multiresistant bacteria in humans and animals.

Key words: resistance, antibiotics, One Health, monitoring, NRL-AMR

Acknowledgement: This work was funded by Ministry of Science, Technological Development and Innovation of Republic of Serbia by the Contract of implementation and funding of research work of NIV-NS in 2024, Contract No: 451-03-66/2024-03/200031.

RECENT TRENDS IN ANTIMICROBIAL RESISTANCE OF BACTERIA FROM LIVESTOCK AND MEAT IN THE NETHERLANDS – FROM A ONE HEALTH PERSPECTIVE

Kees T. Veldman[1*]

[1]National Reference Laboratory for antibiotic resistance in animals, Wageningen Bioveterinary Research, Lelystad, Netherlands

*kees.veldman@wur.nl

Antibiotics are used in animal husbandry to treat bacterial infections. Bacteria can become resistant to antibiotics during treatment, therefore antibiotic stewardship is important. Wageningen Bioveterinary Research (WBVR, part of Wageningen University & Research) studies the occurrence, spread and prevention of antimicrobial resistance (AMR) in bacteria obtained from livestock. Monitoring of trends in antibiotic resistance in livestock and food is determined by analyzing the results generated within an annual standard monitoring program. In practice, this means that bacterial culturing of faecal samples (or meat samples) is performed. The next step is antimicrobial susceptibility testing of indicator bacteria such as *E. coli* as well as zoonotic bacteria such as *Salmonella* and *Campylobacter* in standardised antibiotic panels according to EU legislation. In addition, the collected samples are screened for the presence of bacteria with specific types of resistance that are considered a risk to human health. This includes *E. coli* exhibiting resistance to 3rd and 4th generation cephalosporins (ESBLs) or resistance to carbapenem antibiotics (CPE). Until recently, the resistance pattern of ESBL-producing *E. coli* was measured with a phenotypic susceptibility test. In the past three years, this has been replaced by whole genome sequencing. Research shows that the genetic analysis generates valid information to predict phenotypic resistance. In addition, whole genome sequencing provides much more information, such as relationships between bacteria. This type of information supports knowledge about the potential spread of resistant bacterial clones. The substantial decline in antibiotic use in animals since 2009 is reflected by a trend of decreasing resistance in indicator bacteria, followed by levelling off in more recent years. This effect of decreasing resistance is less clear in zoonotic bacteria like *Salmonella* and *Campylobacter.* Furthermore, the prevalence of ESBLs is low in most animal sectors and CPE remains undetected. Reduction in antibiotic use in livestock has resulted in a clear reduction in resistance of indicator *E. coli.* However, the effect on resistance of zoonotic bacteria from livestock is less clear in NL.

Key words: livestock, trends in resistance, indicator *E. coli*, ESBL

Acknowledgement: The study on antimicrobial resistance in food-animals was primarily financed by the Ministry of Agriculture, Nature and Food Quality through the project 'Antimicrobial Resistance Research in Animals', grant number WOT-01-002-038.

ONE HEALTH APPROACH IN AMR AND ANTIBIOTIC CONSUMPTION CONTROL IN SERBIA –FIVE YEARS OF THE NATIONAL MULTI-SECTORAL COORDINATING GROUP ACTIVITIES

Milica Bajčetić[1*]

[1]Department of Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Belgrade, P.O. Box 38, 11000 Belgrade, Serbia

*mbajcetic@doctor.com

Excessive antibiotic use is a major, among multiple factors, for the development of antimicrobial drug resistance (AMR). As a result of growing concerns, several activities were launched in Serbia over the last decade with the aim of reducing AMR rates. The national multi-sectoral coordinating group was established in 2019 with the aim to adopting a multi-sectoral approach called One Helth framework vital to tackling the complex drivers of AMR across human health, animal health, agrifood systems and the environment. A pioneer step regarding the human antibiotic consumption was the increased control of pharmacies in 2011 and 2015 in order to stop the dispensing of antibiotics without prescription. In addition, in 2015, the first campaign for rational antibiotic use was launched by the Serbian Ministry of Health and implemented through the Second Serbia Health Project funded by the World Bank. Further efforts to apply the latest knowledge regarding appropriate antimicrobial use were made in 2018 when a first national guideline for rational antibiotic use was published. After the campaign, the trend of decreased antibiotic consumption became evident: the data for 2016 and 2017 showed a significant decreases compared to 2015 by a total of 32.79%. We assume that the significant decrease in antibiotic consumption, which is the result of a government campaign, significantly lessened the expected increase in antibacterial resistance due to the excessive antibiotic consumption observed during the COVID-19 years. The structure of antibiotics consumption has dramatically changed during the COVID-19 era. Watch group of antibiotics represented almost 60% of total antibiotic consumption in the 2020 and 2021. Apart from the WHO Europe Antimicrobial Medicines Consumption Network (AMC), Serbia is the part of the Central Asian and Eastern European Surveillance (CEZAR) network. The data regarding antimicrobial medicines consumption and bacterial resistance from Serbia are part of the annual WHO Europe AMC and CEZAR reports. In addition, annual reports for antibiotic consumption in primary health care is available thank to the e- prescriptions IT platform established in 2019. Good insight into the prevalence of hospital-acquired infections (HAI) as well as the most frequent causative pathogens in Serbian hospitals provided us European point prevalence surveys (PPS) on HAI and antimicrobial use in acute care hospitals organized by European Centre for Disease Prevention and Control (ECDC). Although Serbia is not an EU country, we participated in all PPS from 1998 including the current in 2022. In addition, AMR in veterinary practice is monitoring in 12 certificated laboratories in Serbia. The annual data regarding antibiotic consumptions are available for veterinary practice also. Serbia performs well in most policy areas but there is room for further policy action in such as good management and hygiene practices in food processing, biosecurity practices in terrestrial animal production as well as optimising antimicrobial use and infection prevention in human health. One Health training and education is important to promote the understanding of the multifaceted interplay between pathogens, hosts, and the environment.

Key words: AMR, One Helth, antibiotic consumption, PPS, veterinary practice

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THE ECONOMIC COST OF ANTIMICROBIAL RESISTANCE – FAO PERSPECTIVE THE TRADE IMPACTS OF ANTIMICROBIAL MAXIMUM RESIDUE LIMITS IN FOOD: CASE OF SHRIMP EXPORTS TO EU

Diletta Topazio[1*], Spencer Henson[2]

[1]Food and Agriculture Organization of the United Nations, Rome, Italy
[2]University of Guelph, Guelph, Ontario, Canada

*diletta.topazio@fao.org

The global trade of food products is subject to stringent controls, particularly regarding Maximum Residue Limits (MRL) for antimicrobial residues. Despite efforts to harmonize these standards, significant variation persists worldwide, posing challenges for exporters. Border rejections due to non-compliance with MRL are a common occurrence, especially in high-income markets like the EU. Shrimp exports, heavily impacted by these rejections, reflect broader trade dynamics in fish and seafood. Vietnam, India, and China account for a majority of EU rejections, signaling a need for improved production practices. Economic estimates reveal the enduring negative impact of border rejections on shrimp exports to the EU, highlighting persistent compliance challenges. Addressing antimicrobial resistance (AMR) in trade necessitates international cooperation and responsible practices. Organizations like WHO and WOAH advocate for guidelines to balance trade facilitation with AMR mitigation. Harmonizing regulatory standards and promoting responsible antimicrobial use are crucial steps, requiring collaboration between high-income importers and LMIC exporters. Trade serves as a platform for knowledge transfer and capacity-building, fostering sustainable practices and effective strategies to combat AMR globally.

Key words: antimicrobial maximum residue limits, trade, shrimps

DIFFERENT ASPECTS OF HUNGARIAN ANTIBIOTIC USE

Benkő Ria[1,2,3*], Erika Papfalvi[1,3], Mária Matuz[1,2]

[1]Clinical Pharmacy Institute, Faculty of Pharmacy, University of Szeged, H-6725 Szeged, Hungary;
[2]Central Pharmacy, Albert Szent Györgyi Medical Center, University of Szeged, H-6725 Szeged, Hungary
[3]Department of Emergency Medicine, Albert Szent Györgyi Medical Center, University of Szeged, H-6725 Szeged, Hungary

*benkoria@gmail.com

Antimicrobial resistance (AMR) is one of the global threats. Despite other factors, antimicrobial use is a key driver for AMR and rationalising antibiotic use is one of the most important tools to combat AMR. To be able to develop strategies, we need to identify the problematic areas of antibiotic use, with the application of drug utilisation studies. In this lecture we aimed to give insight into the trends of antibiotic use in Hungary with refering to reports and to some recently conducted researches. We also included benchmarkings with other European countries. All of the presented data will be national level. We used different reports (JIACRA, ECDC) and data sources (health insurance data and wholesales data) to quantify antibiotic use. Different outcome measures were applied (tonnes, mg/kg biomass, DDD /1000 inhabitants/day and DDD/100 patient-days, packages/child/year). The distribution of antibiotic use between human and animal health and between different sectors, the evaluation of the scale of use, seasonality patterns and other quality indicators, focusing on special populations (pediatrics) and the impact of the COVID pandemic will be included. In conclusion, we will show that not only tha pattern of antibacterial use is far from ideal in Hungary, but there is a lot of room to decrease the scale of use, despite that some drug specific quality indicators show ideal situation in comparison to other European countries.

Key words: antibiotic utilisation, quality indicators, scale of use, pattern of use

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ANTIMICROBIAL CONSUMPTION IN MONTENEGRO FROM 2009 TO 2022

Snežana Mugoša[1,2*], Lidija Ćizmović[2], Dragana Tomanić[3], Zorana Kovačević[3]

[1]Faculty of Medicine, University of Montenegro, Krusevac, bb, 81000 Podgorica, Montenegro
[2]Institute for medicine and medical devices of Montenegro, Bulevar Ivana Crnojevića, 81000 Podgorica, Montenegro
[3]Department of Veterinary Medicine, Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovica 8, 21 000 Novi Sad, Serbia

*snezana.mugosa@cinmed.me

Antimicrobial agents are crucial in treating human infectious diseases, yet their inappropriate and excessive use poses a significant global health threat by fostering antimicrobial resistance (AMR). To address this challenge, databases and surveillance systems tracking antimicrobial consumption are essential. This study analyzed national sales data of systemic antimicrobials from 2009 to 2022, sourced from the database of the Institute for Medicines and Medical Devices of Montenegro (CInMED). Using the World Health Organization methodology based on the Anatomical Therapeutic Chemical (ATC) Classification System and Defined Daily Dose (DDD), antimicrobial consumption trends were examined. A noticeable decreasing trend in consumption, expressed in DDD/1000/day, was observed from 42.50 in 2009 to 32.40 in 2022, with a significant decrease noted in 2018 (27.21), potentially indicating a turning point in antimicrobial usage. While there was a slight increase in 2021 (32.65), consequence of the covid-19 pandemic, 2022 displayed minor decreases, suggesting a relatively stable overall trend in antimicrobial consumption. In conclusion, these findings underscore the importance of accurate national data in combating antimicrobial misuse and understanding AMR patterns. Furthermore, it emphasizes the crucial role of promoting responsible antimicrobial use to address the growing challenge of AMR and preserve the efficacy of antimicrobial drugs for future generations.

Key words: antimicrobial consumption, antimicrobial resistance, CInMED, Montenegro

FROM 'TWO MEDICINES' TO 'ONE HEALTH' AND BEYOND

Olga Horvat[1*], Zorana Kovačević[2]

[1]Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine Novi Sad, University of Novi Sad, Novi Sad, Serbia
[2]Department of Veterinary Medicine, Faculty of Agriculture Novi Sad, University of Novi Sad, Novi Sad, Serbia

*olga.horvat@mf.uns.ac.rs

Close interactions between human and animals are longstanding but vary by their historical intensity and cultural background. By applying the knowledge of cattle anatomy and physiology gained through their domestiction, care and ritual bull sacrifice to human analogues this led to the origin of human medicine in ancient Egypt. However, human and veterinary medicine diverged during the 19th century and one of the contributory factors to such divergence could be veterinary medicine's close association to livestock and horses, before companion animal medicine gained prominence from the 1970s. Despite their current disconnect, human and veterinary medicine do share commonalities. They share a common body of knowledge e.g. anatomy, disease origins, pathology and physiology. Given animals share many of the same chronic diseases as humans there are shared disease interests e.g. cancer, heart disease etc, with solutions e.g. treatments, often being similar. Bacterial pathogens present health challenges to both human and veterinary medicine, e.g. MRSA, *E. Coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. In terms of treatment, some classes of antibiotics such as the high priority critically important antibiotics) e.g. 3rd and 4th generation cephaloporins, fluoroquinolones and colistin overlap both veterinary and human medicine. Antibiotic overuse and misuse in both the medical and veterinary professions has been a contributory factor to antibacterial resistance (ABR). Compared to non-zoonotic pathogens, zoonotic pathogens are twice as likely to beassociated with newly discovered, emerging, human illnesses. Indeed, of all the infectiousagents that infect humans, approximately 60% are zoonotic. The effective fight against antimicrobial resistance and zoonoses requires the One Health approach. If we are to improve communication between human and veterinary medicine, new strategies are required for the One Health initiative to gain traction. One such strategy to improve communication between human and veterinary medicine is to begin with students. Introducing interdisciplinary group projects into the curricula of medical and veterinary schools would foster teamwork and early networking between veterinary and medical students. The first step was made between the Faculty of Medicine and Department of Veterinary medicine at the University of Novi Sad. We evaluated the knowledge, attitude and practice of students of medicine, dentistry and veterinary who will be the only prospective prescribers of antibiotics in relation to judicious use of antibiotics. Although students of medicine and students of dentistry demonstrated a higher level of knowledge about antibiotic use than students of veterinary medicine, as far as attitude and practices regarding ABR and usage are concerned, there is a significant need for improvement among all three groups of future prescribers. The solution to this problem requires a concerted One Health approach to mitigate future risks to humans, animals and the environment.

Key words: One Health, antibiotics, students

THE ONE HEALTH APPROACH IN THE FIGHT AGAINST ANTIMICROBIAL RESISTANCE

Lejla Velić[1]*, Toni Eterović[1]

[1]University of Sarajevo-Veterinary faculty, Sarajevo, Bosnia and Herzegovina

*lejla.velic@vfs.unsa.ba

Antimicrobial resistance (AMR) encompasses humans, animals, and the environment, hence combating this global problem requires a One Health approach. One Health unifies an approach aimed at optimizing the health of humans, animals, and ecosystems, recognizing that all three factors are interconnected and interdependent. Thus, addressing this global health issue entails a multidisciplinary approach. The One Health approach mobilizes multiple sectors, disciplines, and communities at various levels of society to collectively address threats to health and ecosystems, while also addressing the collective need for healthy food, water, energy, and air, tackling climate change, and contributing to sustainable development. The development of antibiotic resistance is closely linked to the type and amount of antibiotics used. Resistance can emerge even after the initial treatment with antibiotics, underscoring the importance of using antibiotics only when truly necessary, both in humans and animals. Unfortunately, antimicrobial agents are often used in veterinary practice without proper control, frequently for preventive and sometimes for diagnostic purposes. Resistant bacteria can be transmitted and spread from animal to animal. Some bacteria can also be transmitted between animals and humans. Resistant bacteria can be transmitted from the environment to both humans and animals, and vice versa, posing a significant challenge in the fight against antimicrobial resistance. The aim of this presentation is to highlight the role of veterinary medicine and animal health control in the multidisciplinary approach to addressing antimicrobial resistance.

Key words: antimicrobial resistance, One Health, veterinary medicine

COLISTIN: OLD ANTBIOTIC - NEW PROBLEM

Vuk Vračar[1*], Danijela Petrović[2], Gordana Kozoderović[2], Vesna Lalošević[1]

[1]University of Novi Sad, Faculty of Agriculture, Department of Veterinary Medicine, Novi Sad, Republic of Serbia
[2]University of Novi Sad, Faculty of Education in Sombor, Department of Natural Sciences and Management in Education, Sombor, Republic of Serbia

*vuk.vracar@polj.uns.ac.rs

A new promising antibiotic, with significant activity against Gram-negative bacilli, was discovered in Japan at the end of the 40s of the last century. This polypeptide was isolated from bacterium *Paenibacillus polymyxa* (then *Bacillus polymyxa* var. *colistinus*) and named colistin. Just a few years after, it was introduced into clinical practice, but high prevalence of nephro and neurotoxicity was observed. Initially a miracle antibiotic, colistin was consequently abandoned in favor of less toxic antibiotics such as aminoglycosides. For decades its use was limited to the treatment of secondary infections in patients with cystic fibrosis. During the same period, colistin experienced a renaissance in veterinary medicine being widely used for the growth promotion and prevention and control of diseases in animal husbandry, with the swine and poultry industries utilizing around 96% of colistin globally. The consumption of colistin in animals has been increasing since and has been particularly high in developing countries, with China sole reaching 2875 tones in 2014. The rapid rise of multidrug resistance (MDR) and the lack of novel antimicrobials in the past two decades resulted in reintroduction of colistin in human medicine as a last-resort antibiotic, for the treatment of severe infections caused by MDR Gram-negative bacteria. From the aspect of antimicrobial resistance colistin was, for a long time, considered as a safe antibiotic. Until 2015 resistance to colistin has been associated with the non-transferable genome-mediated mutation. Because of its relatively recent reintroduction to clinical practices prevalence of resistance to colistin in human clinical isolates remained low. The alarm went on upon the discovery of plasmid-mediated, mobile colistin resistance (*mcr*) genes indicating the ability of horizontal transmission among bacteria of the same or different species. Today, ten families of *mcr* gene (*mcr-1* to *mcr-10*) are reported in bacteria isolated from different sources worldwide. Recent studies marked livestock as hot spot for amplification and spread of colistin resistance. Orally administered colistin is known to be poorly absorbed in the gastrointestinal tract, thus causing the emergence of colistin resistance through the selective pressure upon the gut microbiota. Furthermore, the excretion of antimicrobial-resistant bacteria into the environment may spread antibiotic resistance between species by direct exposure or through the food chain and the environment. In 2020, the World Health Organization (WHO) reclassified colistin into a category of critically important antimicrobials in human medicine, category B, which means limitation of its use in veterinary medicine in order to mitigate the risk of public health. Recent experience from China indicates a strong causal relationship between the use of colistin in feed and resistance, where the ban on the use of colistin in animals in 2016 led to a sharp decrease in the prevalence of *mcr-1* in pig farms from 45% in 2016 to 19% in 2018. However, Serbia continues to use colistin and annual consumption is measured in tons. So far, *mcr-1* gene was detected in pigs and turkeys which only scratched the surface of its complex epidemiology. Bearing in mind that colistin resistance is present in Serbia and its importance for human medicine, alternatives to colistin in prophylaxys and metaphylahys in animal husbandry should be used in order to reduce the spread of its resistance.

Key words: colistin, antibiotic resistance, One Health

ACTIVITIES OF THE LOCAL AND REGIONAL ONE HEALTH ORGANISATIONS

Sara Savić[1*], Marina Žekić[1], Dalibor Todorović[1], Igor Stojanov[1], Maja Velhner[1]

[1]Scientific Vetrinary Institute "Novi Sad", Novi Sad, Serbia

*sara@niv.ns.ac.rs

One Health is an approach, it is a way of thinking, connecting and sharing information. One Health is not a scientific topic, it is a concept, an integrated, multidisciplinary approach that leads to environmentally sustainable health care systems for humans and animals including ecosystems. One Health evolved during the last decade and in 2023, One Health High-Level Expert Panel (OHHLEP) was created by the heads of the United Nations Food and Agriculture Organization (FAO), the United Nations Environment Programme (UNEP), the World Health Organization (WHO), and the World Organization for Animal Health (WOAH; founded as OIE) to provide technical and scientific advice on One Health issues by the enhanced science-based cross-sectorial, multidisciplinary collaboration. The new definition of One Health was developed by the OHHLEP stating that "One Health is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems. It recognizes that health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and interdependent. The approach mobilizes multiple sectors, disciplines and communities at varying levels of society to work together to foster well-being and tackle threats to health and ecosystems, while addressing the collective need for healthy food, water, energy, and air, taking action on climate change, and contributing to sustainable development. One Health approach can be related to many topics, but one that stands out lately is without doubt Antimicrobial Resistance (AMR). AMR is a global problem, it affects both humans and animals, but also environment, because of the excessive use of antibiotics on all levels. One Health approach is the only way leading to resolution. Local and regional activities related to One Health approach and also organizations working under One Health concept will be described.

Key words: One Health, organizations, local, regional

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INFLUENCE OF CROSS-CONTAMINATION OF ANIMAL FEED WITH ANTIMICROBIALS ON INTESTINAL MICROBIOME

Ewelina Iwan[1], Ewelina Kamińska[1], Anna Grenda[2], Małgorzata Olejnik[3*]

[1]National Veterinary Research Institute, Pulawy, Poland
[2]Medical University of Lublin, Lublin, Poland
[3]Nicolaus Copernicus University, Torun, Poland

*malgorzata.olejnik@umk.pl

Exposure of animals to antimicrobials can generate various adverse effects. Apart from antimicrobial resistance (AMR), it could result in changes in the intestinal microbiome, morphology and functions of intestines, immunological status, and antibiotic residues. In the European Union, 24 different antimicrobial compounds may be administered with medicated feed, which poses a risk for feed cross-contamination and constant exposure of animals to low doses of antimicrobials. European Food Safety Authority has assessed risk using the Feed Antimicrobial Resistance Selection Concentration (FARSC) model. Unfortunately, this assessment does not include risks other than AMR and growth promotion. Moreover, because of made assumptions, complex interdependencies occurring *in vivo* cannot be taken into account. That is why we have decided to perform an animal study to verify the effects of antimicrobials from different classes on broiler chickens. Six antibiotics (colistin, doxycycline, flumequine, thiamphenicol, tiamulin, tilmicosin) were tested and compared to the control group. Antibiotics were given to broiler chickens in feed during the whole rearing period at doses close to 1% of the maximum approved concentration. Contents of the caecum were sampled after one, three, and five weeks (n=8/group). DNA was isolated, and custom-made libraries were sequenced (Illumina MiSeq V3, 2x300). Metataxonomic analysis and diversity metrics calculations were performed using qiime2. Broilers' microbiomes changed according to the age of the broilers and specific antibiotics. The number of ASVs (Amplicon Sequence Variants) increased with subsequent sampling in each group. The diversity of the microbiome was the highest in the control group at five weeks; it was significantly decreased at earlier steps of chicken development. The exposure to doxycycline, thiamphenicol, and tilmicosin induced changes in diversity already in the first week. At five weeks, there were significant distances between the control and all study groups, except flumequine. Regarding the composition of the microbiome, the strongest effects were observed at three weeks. Thiamphenicol disturbed it the most, increasing the abundance of mostly aerobic bacteria (*Staphylococacaeae*, *Enterobacteriaceae*, *Enterococaceae*, *Erysipelotrichaceae*) and reducing the percentage of *Ruminococeae*, *Christensenellaceae*, and *Clostridia* UCG014. Tilmicosin induced similar but less pronounced changes, affecting additionally *Oscillospiraceae* and *Butyriciococcaceae*. Doxycycline, colistin, and flumequine affected the abundance of single bacteria families. Although age influenced the microbiome more than exposure to antimicrobials, significant changes were observed in almost every group. Considering the microbiome's importance in many processes, including immunological responses, this could affect animal health and welfare. The presented data will be interpreted together with other results obtained within our larger, multidirectional project entitled "Hazards related to cross-contamination of feed with antibiotics".

Key words: 16S rRNA sequencing, microbiome, feed cross-contamination, broiler chicken

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ASSOCIATION OF AMR IN *E. COLI* FROM CALVES RAISED FOR MEAT PRODUCTION WITH HUSBANDRY SYSTEM

Bernd-Alois Tenhagen[1*], Carolina Plaza-Rodriguez[1], Katja Alt[1], Mirjam Grobbel[1]

[1]German Federal Institute for Risk Assessment, Berlin, Germany

*Bernd-Alois.Tenhagen@bfr.bund.de

Antimicrobial resistance has been described as a relevant topic in calf husbandry. Bacteria isolated from calves are more frequently resistant to antimicrobials than isolates from other groups of cattle such as dairy cows of beef cattle at slaughter. However, in Germany and other countries calves are raised in diverse production systems and it remains to be elucidated if and how this has an effect on the level of AMR observed. In the framework of a monitoring programme we collected faecal samples from two age groups of calves raised in the herds where they had been born (i.e. dairy herds or suckler herds) and from calves that were raised in specialised units (beef or veal farms) after being traded. *Escherichia coli* were isolated from the samples and tested for their resistance to 15 antimicrobials using broth microdilution as prescribed by Commission implementing decision (EU) 2020/1729. Three hundred thirty seven pairs of isolates from different farms were included in the analysis. Overall, the majority of isolates (54.3 %) were susceptible to all 15 antimicrobials tested. Proportions of resistant isolates were higher in younger calves (54.9 %) as compared to older calves (36.5 %). Calves in dairy and suckler herds that had not been traded had lower proportions of resistant isolates (22.9 and 18.6 %, respectively) than calves in beef and veal farms that had been traded (64.7 and 63.3 %, respectively). AMR was highest to tetracycline, sulfamethoxazole, ampicillin, trimethoprim and chloramphenicol. Resistance was absent to meropenem, colistin and amikacin. Resistance was below 10 % for the other antimicrobials. Differences between the four groups were observed for all the included antimicrobials except for those were resistance was fully absent. Herd size was also positively associated with the proportion of resistant isolates for most of the substances. Our results indicate that the level of AMR in *E. coli* from calves is associated with type of husbandry (especially the fact that they have been traded), with the age of the animals (lower resistance in older calves) and with herd size (more resistance in larger units). Further studies need to show whether these differences are purely a consequence of different levels of antimicrobial use in the systems or if other management aspects also contribute to these differences. Moreover, risk factors for AMR (and AMU) within the systems need to be analysed to reduce the need of treatment and consequently the risk of AMR.

Key words: calf, trade, resistance, husbandry, *E. coli*

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ANTIMICROBIAL RESISTANCE OF *PSEUDOMONAS* SPP. ISOLATES FROM MILK

Radoslava Savić Radovanović[1*], Marija Pajić[2], Nemanja Zdravković[3]

[1]University of Belgrade, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Belgrade, Serbia
[2]University of Novi Sad, Faculty of Agriculture, Department of Veterinary Medicine, Novi Sad, Serbia
[3]Scientific Institute of Veterinary Medicine of Serbia, Belgrade, Serbia

*mimica@vet.bg.ac.rs

The genus *Pseudomonas* is one of the most diverse bacterial genera, containing ubiquitous and metabolically versatile bacteria. Considering its complexity, the antimicrobial resistance problem must be discussed multidisciplinary. In accordance with the "One Health" approach it is not wise to separate the antimicrobial resistance of *Pseudomonas* spp. from its biology and milk hygiene/technological problems which it can cause. *Pseudomonas* members are psychotrophic Gram negative rods, adapted to various conditions and therefore are found in a wide range of niches, such as soil, water, plants, animal tissues, foods, etc., thanks to their complex enzymatic systems. Due to their simple nutritional requirements and rapid growth, they have the ability to survive under a variety of environmental conditions and are frequently implicated in the degradation and spoilage of a wide range of foods. *Pseudomonas* spp. produce extracellular proteolytic and lipolytic enzymes during their growth in raw milk and these enzymes remain active after pasteurisation, reducing the quality of stored pasteurised milk, even though the bacteria have been destroyed. The presence of *Pseudomonas* spp. in refrigerated raw milk is most likely due to contamination during and/or after milking, with the milking equipment usually being responsible for the greatest contamination, since they are not considered to be a major component of the microbiota of the bovine udder. *Pseudomonas* spp. occasionally cause mastitis in dairy cattle. Infections may be epidemic, sporadic, or endemic within a herd. Epidemics of *Pseudomonas* mastitis have been associated with contaminated wash hoses and water supplies. Water tanks, hoses, and pipeline connectors should be suspected as harbouring *Pseudomonas* spp. whenever cases of this type of mastitis are diagnosed. Reports of contaminated vials, cannulas, or syringes used for the administration of antibiotics being the sources of *Pseudomonas* spp., whose multiplication was possible in the presence of antibiotics or sublethal concentrations of sanitizers, can be found in the literature. Previously reported results showed that this microorganism occurred in raw bulk milk tanks. Hygiene criteria for primary food production, set in the European Regulation, as well as in that of the Republic of Serbia, do not include detection of *Pseudomonas* spp. Their ability to form biofilm facilitates the development of resistant strains phenotypically expressing glycocalyx or slime. Further investigations should focus on examining biofilm forming ability in *Pseudomonas* isolates from solid surfaces in dairy production. In conclusion, *Pseudomonas* spp. might contaminate raw food of animal origin, consequently leading to spoilage and may be considered as a reservoir of *Pseudomonas* spp. resistome. Compared to *Pseudomonas* studies in human medicine, where antimicrobial resistance is often a causative agent of nosocomial infections, food isolates do not appear to create resistance to large numbers of antimicrobials.

Key words: antimicrobials, dairy production, milk, *Pseudomonas* spp., resistance

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MONITORING OF ANTIMICROBIAL DRUG RESIDUES IN MILK FOR FURTHER PROCESSING

Marija Pajić[1a*], Radoslava Savić Radovanović[1a], Ksenija Čobanović[1b], Suzana Vidaković Knežević[3], Jelena Vranešević[3], Čaba Čorba[4], Zorana Kovačević[1a], Jovan Stanojević[1a], Miodrag Radinović[1a]

[1]University of Novi Sad, Faculty of Agriculture, [a]Department of Veterinary Medicine and [b]Department of Animal Husbandry, Novi Sad, Serbia
[2]University of Belgrade, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Belgrade, Serbia
[3]Scientific Veterinary Institute "Novi Sad", Department of Food Safety, Novi Sad, Serbia
[4]Provincial Secretary for Education, Regulations, Administration and National Minorities – National Communities, Novi Sad, Serbia

*marija.pajic@polj.edu.rs

In raw milk production, mastitis represents one of the most important diseases and a major cause of economic loss in the dairy industry. During the last decades, antimicrobial drugs have been used daily on dairy farms. Monitoring of antimicrobial drug residues (ADR) in raw milk is required by Food Safety Law in Republic of Serbia. The presence of ADR in milk is undesirable from several aspects. From the human health point of view, allergic reactions, even anaphylaxis or sensitization to the drug, are possible. Also, the microbiome of milk consumers, exposed to low concentrations of the drug, can develop resistance to that drug, which later can be transferred to other, related microorganisms. In the dairy production, ADR in raw milk could delay or inhibit fermentation and curdling, due to the effect of ADR on the starter culture. According to the current regulations, the trade of raw milk containing ADR above the permitted, prescribed limit is forbidden. However, ADR are occasionally found in raw milk. In accordance with perspective approach to ensure food safety and the longitudinal integrated safety assurance (LISA), it is necessary to monitor ADR in raw milk. In this research, the most common causes of ADR in raw milk were examined. Data were collected from the laboratories of the Faculty of Agriculture in Novi Sad and the Scientific Veterinary Institute "Novi Sad", as well as from dairy farms and dairy facilities which cooperate with those laboratories. In the dairies, when buying raw milk from farms, raw milk must be examined for ADR. Also, according to Good Manufacturing Practice, in some dairies participating in this research, the yogurt test is used to select raw milk for the production of fermented milk products. In most cases, when the presence of inhibitory substances in raw milk was proved, ADR were found. It is known, that excretion of antimicrobials depends on drug amount and application method, chemical characteristics of drug, physiological status of treated animal, as well as the nature of the pathological process. In all studied cases, the occurrence of ADR in the bulk tank milk was the result of an oversight or a mistake. The most common causes of ADR occurrence in raw milk were: presence of ADR in raw milk at the beginning of lactation in cows that were treated during the dry period; the lack of information on the treatment of newly acquired cows; cases when one quarter of udder is treated, and the milk from the other quarters is milked in the bulk tank. In some cases, unprofessional, negligent and irresponsible use of antimicrobials was found to be the reason for ADR findings in raw milk. In conclusion, the improvement in raw milk production management and constant monitoring of ADR in raw milk is necessary in order to protect milk safety and public health.

Key words: milk safety, dairy production, antibiotic residues

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MYCOTOXINS ESCALATION CHALLENGES IN THE LIGHT OF CLIMATE CHANGE

Jovana Kos[1*], Ivana Čabarkapa[1], Bojana Radić[1], Radmila Radović[1], Pavle Jovanov[1], Ljubiša Šarić[1], Bojana Šarić[1]

[1]Institute of food technology in Novi Sad, University of Novi Sad, Novi Sad, Serbia

* jovana.kos@fins.uns.ac.rs

Mycotoxins, toxic secondary metabolites produced by certain fungi, pose significant threats to human and animal health, as well as to the economy, agricultural productivity, and social well-being. They are recognized for their ability to induce a range of adverse health effects, from acute poisoning and immunosuppression to carcinogenicity and developmental abnormalities. While numerous factors influence mycotoxins synthesis, recent research has highlighted the significant impact of climate change effects, such as drought conditions, rising temperatures, and changes in rainfall patterns, on fungal growth and mycotoxins occurrence. In the past decade, there has been a concerning escalation in mycotoxins occurrence in certain European regions, affecting various agricultural products, particularly cereals. This trend is strongly associated to climate change. Cereals play a crucial role as primary ingredients in numerous food and feed products, posing a significant risk to the further contamination of the food and feed supply chain. The recent studies indicate that the percentage of contaminated agricultural commodities with mycotoxins is significantly higher than the previously reported 25%, and now very often exceeds 80%. Moreover, the heightened occurrence of mycotoxins, along with the identification of new mycotoxins, is often attributed to both the improved sensitivity of analytical methods and the impact of climate change. Additionally, it should be noted that the implementation of advanced analytical methods of the new generation enables the detection of mycotoxins at significantly lower concentrations than previously possible. This directly contributes to the increase in the percentage of contaminated samples, and it is often observed that some cereal samples are contaminated with several dozen different mycotoxins. Further, numerous scientific studies have confirmed the influence of climate change on the escalation and variations in mycotoxins occurrence and contamination levels. Addressing the challenges posed by the escalation of mycotoxins in the context of climate change requires a multifaceted approach. Strategies for mycotoxins management encompass pre-harvest interventions and post-harvest measures. Additionally, robust regulatory frameworks and surveillance systems are critical for ensuring compliance with safety standards and monitoring mycotoxins levels in food and feed supplies. In conclusion, tackling the escalation of mycotoxins in cereals driven by climate change presents complex challenges for food and feed safety and security. Dealing with these challenges necessitates coordinated efforts across multiple sectors, including agriculture, production, regulation, and public health. By implementing comprehensive strategies for mycotoxins management, it is possible to reduce the risks posed by these toxic contaminants and safeguard the integrity of the global food and feed supply chain.

Key words: mycotoxins, climate changes, global warming, cereals

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ANTIMICROBIAL RESISTANCE IN FOOD AND FOOD PRODUCING ANIMALS IN THE REPUBLIC OF MACEDONIA

Marija Ratkova Manovska[1*], Katerina Dodovska Blagoevska[1], Dean Jankuloski[1]

[1]University Ss Cyril and Methodius, Faculty of veterinary medicine, Lazar Pop – Trajkov 5-7, 1000 Skopje, Macedonia

*ratkova.marija@fvm.ukim.edu.mk

In order to get an idea of the status of antimicrobial resistance in bacterial isolates obtained from food of animal origin as well as in isolates obtained from the digestive system of animals from slaughterhouses, in EU 2011 was determined a plan that would monitor the antimicrobial resistance in zoonotic and commensal bacteria. For the harmonisation of the plan, the EU Commision addopted legislation known as Commission Implementing Decision of 12 November 2013 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2013/652/EU) and it was implemented in EU countries from 1.1.2014. According Annex A of this Decision, it is necessary to monitor the following bacteria: indicator commensal *E. coli*, indicator commensal *E. faecalis* and *E. faecium, Salmonella spp., Campylobacter jejuni, Campylobacter coli* and ESBL- or AmpC- or carbapenemase-producing *Salmonella spp.* and *E. coli.* The laboratory protocols for all analyses were created and verified by the European Reference Laboratory for Antimicrobial Resistance at the Technical University of Copenhagen, Denmark - DTU (Danmarks Tekniske Universitet). In the Republic of North Macedonia, Decision 652/2013 was first brought to force with the Antimicrobial Resistance Program of FVA from 2017, according the Official Gazette of R.M. number 49 of April 24, 2017. With this, our country, although it is not a member state of the EU, has been implementing the plan from 2017 until today, with the help of the accredited methods at the Laboratory for Microbiology of Food and Feed at FVM, making it unique among the non-EU Balkan countries where the monitoring is carried out and reported in EFSA annual reports.

Key words: antimicrobial resistance, food, food producing animals

FOOD SAFETY ISSUES RELATED TO ANTIBIOTIC RESIDUES IN AQUACULTURE

Dragana Ljubojević Pelić[1*], Miloš Pelić[1], Nikola Puvača[2], Jasna Kureljušić[3], Sandra Jakšić[1], Ana Gavrilović[4], Milica Živkov Baloš[1]

[1]Scientific Veterinary Institute "Novi Sad", Novi Sad, Serbia
[2]Department of Engineering Management in Biotechnology, Faculty of Economics and Engineering Management in Novi Sad, University Business Academy in Novi Sad, Novi Sad, Serbia
[3]Scientific Institute of Veterinary Medicine of Serbia, Belgrade, Serbia
[4]University of Zagreb, Faculty of Agriculture, Zagreb, Croatia

*dragana@niv.ns.ac.rs

Aquaculture is a rapidly expanding food industry sector and one of the most sustainable sources of animal protein. Similar to agriculture, aquaculture is believed to use a lot of antimicrobials, which are among the most well-known and frequent chemical contaminants that find their way into the environment and consequently the food chain. The present work aims to provide an overview on the application, residues, regulations, possible health risks to humans associated with antibiotics used in aquacultureand some recommendations. Treatments of fish are usually regarded as metaphylactic and antibiotics are added to feed or, less frequently, directly to the water. Although their primary function is to inhibit the growth of microbes, these compounds have also long been exploited as growth promoters in fish production. There are a number of reasons why residues can be found in edible tissues, such as usage of medications against recommended dosages or instructions, disregard recommended withdrawal times, administer too many antibiotics, use contaminated equipment, or neglect to properly clean equipment used to mix or administer medications or inadvertently consuming medicated feeds. It is extremely difficult to collect trustworthy statistics on antibiotic consumption in majority of countries worldwide, which makes determining the exact extent of the risk of antibiotic residue in aquaculture extremely challenging. On the other side, in the majority of European countries, the use of antimicrobials is closely limited by law. Antimicrobial residues have significant negative effects on public health, including the emergence of drug resistance to antimicrobials, cancer, teratogenicity, mutagenicity, changesin standard intestinal flora, hypersensitivity reactions and others. In order to protect the consumer's health it is necessary that antibiotic residues in products delivered from aquaculture be controlled. The usage of antimicrobials should be reduced in order to decrease the concentration of residue in edible tissues and also to slow down the development of antimicrobial resistance. Also, the quality of water bodies introduced in aquaculture should be regularly controlled and proper preventions strategies should be developed. National and international regulatory frameworks on the sales, usage, and quality of antimicrobial agents are necessary for better management and control over the use of antimicrobial agents in aquaculture as well as for effective prevention. Food safety and consumer protection depend on the availability of straightforward and trustworthy screening methods for identifying antimicrobial residues in aquatic animal tissue.Alternative approaches have concentrated on boosting fish immunity and preserving a pond's and fish's health microbiome. Fish can currently be treated with a variety of immune stimulants, such as vaccination, immune stimulants, probiotics, prebiotics, herbal medicines, essential oils, bacteriophages, albeit there is still relatively little information on how effective they are. Proper handling and distribution by dealers, veterinary supervision of farmers' administration of antibiotics with observance of withdrawal periods prior to slaughter, and clear instructions from medication makers are all necessary for the responsible use of antibiotics.

Key words: aquaculture health management, antimicrobials, food safety, veterinary drug residue

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ALTERNATIVE STRATEGIES IN ANIMAL FEED PRODUCTION TO COMBAT ANTIMICROBIAL RESISTANCE

Nedeljka Spasevski[1*], Ivana Čabarkapa[1], Danka Dragojlović[1], Jelena Vujetić[1], Slađana Rakita[1], Olivera Đuragić[1], Zorica Tomičić[1]

[1]University of Novi Sad, Institute of Food Technology Novi Sad, Serbia

*nedeljka.spasevski@fins.uns.ac.rs

Antibiotics have been employed in the animal industry for over five decades. While their usage has advantages, inappropriate use has led to certain issues, such as the development of antibiotic-resistant bacteria and the presence of drug residues in animal products. The existence of antimicrobial residues in food animal products, such as chicken meat and milk, points to widespread antimicrobial use in the production of food animals. The prohibition of dietary antimicrobial agents has sparked significant interest in the utilization of probiotics, prebiotics, and synbiotics to produce safe food while enhancing gut health. Incorporating beneficial probiotics (*Lactobacillus* and *Bifidobacterium*) into animal feed can improve digestion, enhance nutrient absorption, and inhibit the growth of harmful bacteria. By adding non-digestible fibers (e.g., fructooligosaccharides), the growth and activity of beneficial microorganisms in the intestines can be selectively stimulated, while combining probiotics and prebiotics can maximize the benefits of both strategies, supporting a balanced and resilient gut microbiome. Another natural alternative to antibiotics is the inclusion of phytogenic additives such as essential oils, herbs, and spices, which possess antimicrobial properties. Additionally, recent research has shown that the use of fermented oilseed meals in animal nutrition positively influences intestinal morphology, gut microflora, and immune status of animals. Solid-state fermentation is a bioprocessing approach that has the potential to convert agricultural by-products and crops into products that hold greater value, are enriched with nutrients, and promote sustainability. Peptides derived from solid-state fermented oilseed meal possess antimicrobial properties and can disrupt the integrity of microbial cell membranes, leading to the destruction of pathogens. Antimicrobial peptides (AMPs) are low-molecular-weight molecules exhibiting extensive biological activity against bacteria, viruses, and fungi. AMPs have emerged as a promising strategy due to their diverse biological activities, offering a potent means to combat the development of resistant microorganisms. By incorporating beneficial compounds into animal feed, it is possible to support the health and resilience of animals, reducing the need for antibiotics and mitigating the development of antimicrobial resistance.

Key words: fermentation, oilseed meal, antimicrobial peptides, feed

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THE IMPACT OF FARM BIOSECURITY ON ANTIMICROBIAL USAGE IN LIVESTOCK PRODUCTION

Jeroen Dewulf[1*]

[1]Department of Internal medicine, Reproduction and population medicine, Faculty of Veterinary Medicine, University of Ghent, Belgium

*Jeroen.Dewulf@ugent.be

Limited and judicious antimicrobial usage (AMU) is pivotal in preserving the efficacy of human and veterinary medicine against infections. Farm biosecurity stands out as a promising strategy to curtail non-judicious AMU and uphold animal health, production, and welfare amidst the scarcity of antimicrobial alternatives. This scoping review scrutinizes the influence of farm biosecurity on AMU in livestock systems and provides recommendations. Peer-reviewed manuscripts from 2001 to 2022 were assessed using the PRISMA framework via PubMed, Scopus, and Science Direct databases. Twenty-seven studies met the inclusion criteria, focusing on the impact of farm biosecurity or management practices on AMU at the herd/farm level in quantitative/semi-quantitative terms. Conducted across 16 countries, with a majority (74.1%) from 11 European nations, the studies predominantly targeted pig (51.8%) and poultry (25.9%) farms. Findings revealed complex interactions among biosecurity measures, farm characteristics, farmer attitudes, and access to animal health services influencing AMU. Approximately 51.8% of the studies demonstrated a positive association between farm biosecurity and reduced AMU, while 18.5% indicated that enhancing farm management practices correlated with decreased AMU. Coaching and awareness programs among farmers were identified as potential avenues for reducing AMU. Moreover, one economic assessment underscored biosecurity practices as a cost-effective means to diminish AMU. However, a subset of studies (18.5%) indicated an uncertain or spurious relationship between farm biosecurity and AMU. This review advocates for bolstering the concept of farm biosecurity, particularly in lower- and middle-income countries, and underscores the necessity for strengthening evidence on the association between farm biosecurity and AMU in region- and species-specific farm settings.

Key words: antimicrobial usage, biosecurity, livestock

BIOSECURITY IN POULTRY PRODUCTION IN ITALY

Alessandra Piccirillo[1*], Giuditta Tilli[1], Francesco Galuppo[2], Guido Grilli[3], Andrea Laconi[1]

[1]Department of Comparative Biomedicine and Food Science, University of Padua, Vialedell'Università 16, Legnaro, 35020 Padua, Italy
[2]Unità Locale Socio-Sanitaria (ULSS) 6 Euganea, via Enrico degli Scrovegni 14, 35131 Padua, Italy
[3]Department of Veterinary Medicine and Animal Science, University of Milan, Via dell'Università 6, Lodi, 26900 Lodi, Italy

*alessandra.piccirillo@unipd.it

In poultry production, interventions to prevent and/or control the introduction and the spread of pathogens, such as AI viruses, *Salmonella*, etc. rely mainly on the proper implementation of biosecurity measures (BMs). However, although the importance of implementing biosecurity is well recognized, its compliance is not always optimal. Therefore, it becomes essential to identify and implement the best supporting measures (SMs) to improve biosecurity. As partner of the EU thematic network - Networking European poultry actors for enhancing the compliance of biosecurity measures for a sustainable production (NetPoulSafe) - Italy has conducted several activities aimed at collecting, selecting, testing, and sharing the best SMs to help poultry workers (mainly farmers) in improving biosecurity compliance in farms. Here, we report the results of a survey carried out to assess the implementation of BMs according to poultry stakeholders' perception and the efficacy of selected SMs, such as coaching of farmers, to improve biosecurity compliance in Italian poultry farms. To collect information on BMs implementation, questionnaires were administered to 67 stakeholders (37 advisors and30 farmers) of different poultry production sectors. An overall high level of BMs implementation, *i.e.* 75% (67% and 81.1% according to advisors and farmers, respectively), was detected, with the breeder sector showing the highest level of implementation (85.7%). BMs highly implemented (>70%) included, for example, most of the procedures related to the movement of people (*i.e.*, personnel, visitors, and teams) on the production site; presence of biological vectors (*i.e.*, backyard animals, rodents, and wild birds); cleaning and disinfection (*i.e.*, vehicle's wheels, and house, material, feed silo, and drinking water pipeline between cycles); flock traceability (*i.e.*, flock's register) and health surveillance (*i.e.*, vaccination, clinical signs and mortality monitoring); storage of feed and dead animals. Conversely, BMs scarcely implemented regarded, for example, the circulation of people (*i.e.*, specific clothes and shoes for the chicks' deliverer) on the production site and the microbiological screening after the cleaning and disinfection procedures of the poultry house. To improve biosecurity compliance, 26 pilot farms (PFs) of different poultry production sectors were recruited for a minimum period of 6 months. For each farm, the farmer attitude towards change and the farm biosecurity were quantified using ADKAR-Partage and Biocheck. UGent tools, respectively, at the beginning and the end of the validation phase. During coaching, several different actionplans were agreed by the farmers to implement in their farms, including, for example, improvements of the house hygiene lock (31.8%) and bacterial auto-control after cleaning and disinfection procedures (18.1%). During the study period, anincrease of thefarmer attitude towards biosecurity improvement and the biosecurity score of the farm was also achieved. Despite an overall good level of biosecurity, findings of the survey were useful to identify gaps in biosecurity while improvement strategies to fulfil biosecurity gaps present in the Italian poultry production.

Key words: biosecurity, poultry, farmers, advisors, coaching

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CURRENT STATUS OF BIOSECURITY MEASURES IN THE PIG PRODUCTION SECTOR IN SERBIA

Jasna Prodanov-Radulović[1*], Jelena Petrović[1], Dejan Bugarski[1],Siniša Grubač[1], Marina Štukelj[2]

[1]Scientific Veterinary Institute "Novi Sad", Novi Sad, Republic of Serbia
[2]University of Ljubljana, Veterinary Faculty, Ljubljana, Republic of Slovenia

*jasna@niv.ns.ac.rs

The biosecurity in pig production is currently the number one issue being frequently discussed in the European research society. However, when analysing biosecurity measures, it should be considered that certain European regions have own pig production sector specifics. In the Western Balkan region the existence of highly variable pig farming system has been reported earlier. Considering the pig production sector in Serbia, the main and highly important difference comparing the European member countries can be recognised in holdings classification, definition of commercial activity and general sector structure. The aim of this study is to present the current biosecurity challenges related to the structure and characteristics of pig production sector in Serbia. Additionally, the results from biosecurity questionnaires done by field epidemiologists during the 2021-2023 are presented. In total sixty-fivesmall-scale and/or backyard pig farms and ten industrialpig holdings were analysed in this study in the southeast and north regions in Serbia. Farms visits and biosecurity control checks were related to African swine fewer (ASF) outbreaks as a part of official epidemiological investigation. Collected data regarding biosecurity measures were divided into 25 main categories and additional subcategories that are considered present or absent biosecurity risk factors/indicators for introduction of ASF to the farm. Shortly, in all small scale and backyards farms, except five, other domestic animal species (sheep, goats, cattle, poultry) were present. Moreover, home slaughtering was practise in 85% of extensive farms and in the all backwards natural mating was used. In some districts, farmers confirmed close contact of domestic pigs with wild boars or have seen wild boars nearby the premises (35.62%). Swill feeding (i.e. feeding with kitchen waste) was a part of farmer's routine in 40.77% of examined small scale and backyard farms. Additionally, most of the extensive farms used locally produced fresh mowed grass to feed their livestock. Considering the results of biosecurity checks in industrial farms, some critical points in external biosecurity for most of farms were identified: different transport vehicles entering the farm perimeter and lack of adequate sanitary locks for employees. Most importantly, farm workers are in close contact with backyards and pose a foremost biosecurity risk for any disease introduction. It can be concluded the implementation of biosecurity measures in extensive pig sector in Serbia are currently at the very low level, especially when considering external biosecurity. It should be noted that in Serbia biosecurity measures for pig production are not officially required by law and are given only in the form of general recommendations. Identifying biosecurity challenges and potential risky human-related activities is essential for developing effective biosecurity programs for pig production sector in Serbia.

Key words: biosecurity meausres, pig production sector, Serbia

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ANTIMICROBIAL THERAPY: ADVANCES AND CHALLENGES FACING VETERINARY PRACTITIONERS IN BULGARIA

Aneliya Milanova[1]

[1]Department of Pharmacology, Animal Physiology, Biochemistry and Chemistry, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria

*aneliya.milanova@trakia-uni.bg

During the last decades a lot of effort has beenputon optimizing antimicrobial therapy in veterinary medicine. Several institutions, including the European Medicines Agency, the European Food Safety Authority, EU Vet Castand National Food Safety Agencies, have been involved in developing asuccessful strategy for the use of antimicrobial drugs. The lesson learned from the application of antibiotics in veterinary practice is that the interrelationship between humans, animals, and environmental health is strong and needs to be better understood. Veterinarians bear the highest responsibility for keeping animals healthy and for the responsible use of antibiotics. Decreasing the risk of selecting resistant pathogens is closely related to restriction of any non-essential use of antibiotics. Good practices in the farm animal sector include improvement of animal husbandry and feeding regimesto protect animal health and welfare. It has been widely documented in the scientific literature that the health status of animals and the proper immune system functioning depend on the gastrointestinal barrier and a suitable microbiome. Traditionally, probiotics and prebiotics as well as feed additives based on essential oils are applied. New compounds such as postbiotics and plant extracts are under investigation. Due to their antimicrobial activity, herbsare recognized as a possible alternative in maintaining gut health. More attention is paid to the quality of feed and methods of feed processing and addition of adsorbents to decrease the absorption of mycotoxins. The adoption of antibiotic categorization and promotion of rules for their use in veterinary practice have been recognized as important step inreducing antimicrobial consumption. This strategy brings together the Bulgarian Food Safety Agency (BFSA), the Commission for Drug Registration, veterinary professional unions, and practitioners. The responsibilities and the necessity of communication between these institutions and pharmaceutical companies are recognized, as up-to-dateinformation about the efficacy of older antibiotics is urgently required, along with reconsideration of dosing regimens. The Faculty of Veterinary Medicine plays a leading role by participating in scientific programs and scientific networks such as CA 18217 ENOVAT and CA 22109 Medicinal plants for animal health care, along with representative from the authority such as BFSA. However, challenges still exist: there is no network between the laboratories for susceptibility testing; the pharmacovigilance system is not recognized by veterinarians as a powerful tool, and Bulgarian veterinary legislation needs to quickly reflect EU legislation. There is also a notable gap in communication among physicians, plant specialists, and veterinarians regarding antimicrobial resistance, leading to fragmented solutions to the problem. The introduction of Regulation 2019/6 aims to facilitate online trade of veterinary drugs, including antibiotics, and harmonize the market for these products within the European Union (EU). Easier access of the veterinarians to this market creates new environment that requires responsible prescription and use of antibiotics. Enhanced communication among veterinarians from the Balkan region could have a positive impact on advancements in the prevention and treatment of bacterial diseases in animals.

Key words: antimicrobial therapy, Bulgaria, veterinary practice

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PRUDENT ANTIMICROBIAL USE ON THE POULTRY FARMS IN SERBIA

Marko Pajić[1*], Slobodan Knežević[1], Dalibor Todorović[1], Zoran Ružić[2], Bojana Prunić[1], Biljana Đurđević[1]

[1]Scientific Veterinary Institute "Novi Sad", Novi Sad, Serbia
[2]Department of Veterinary medicine, Faculty of Agriculture, University of Novi Sad, Serbia

*markopajic@niv.ns.ac.rs

The use of antibiotics on poultry farms is of great importance, due to the solution of problems related to the occurrence of various diseases of bacterial etiology. If the drug is administered in the prescribed manner, at the appropriate time, and at the appropriate location, the success of the therapy should be great. It is characteristic of poultry production that individual therapy is not carried out on farms. When clinical symptoms of a disease appear, therapy is carried out in the whole flock. This is of great importance from the aspect of the use of antibiotics, and it is a very important decision of the veterinarian who prescribes the therapy, to assess whether it is needed or not and with which antibiotic to implement it. When choosing an antibiotic, it is best to rely on a previously conducted bacteriological analysis, i.e. antibiogram. If we need to apply an antibiotic quickly, before obtaining an antibiogram (for acute and peracute diseases), we make a decision on the choice of antibiotic based on the observed clinical symptoms, pathological findings, history, and previous experience. In most cases, this approach gives a good result, however, in some cases, after receiving a bacteriological analysis, it is necessary to change the therapy, which often happens in practice. In addition to all these prerequisites for starting antibiotic therapy, preventive use of drugs is also present in practice. This implies giving the medicine before the onset of the disease, without observed clinical symptoms, without the presence of the disease. This study aimed to highlight the importance of the prudent use of antibiotics. For this purpose, a survey was conducted on 45 poultry farms in Serbia. Broiler chicken flocks, fattening turkey flocks, broiler parent flocks, layer parent flocks, and layer hen flocks are included in the analysis. The questions were designed to analyse the use of antibiotics, i.e. whether they are applied in a targeted or preventive manner. The results showed that 68.8% (31/45) of farms use antibiotics for preventive purposes. Out of 31 of them, 24 were found to be predominantly treated with enrofloxacin (77.4%), and 7 farms were dominated by amoxicillin (22.6%). Both antibiotics were most often applied during the first week of the chick's life, and the second dominant period of application was at the end of the 4th week of the chick's life. In that period, amoxicillin was most often used. Also, in addition to these two antibiotics, colistin, doxycycline, flumequine, and trimethoprim+sulfamethasone are dominantly used. Fourteen farms were found to have antibiotics in storage, but do not apply them until there are clinical indications for it. The storage of antibiotics exists only for peracute and acute cases, otherwise, antibiotics are not used until an antibiogram is obtained. Also, on these farms, importance is attached to the use of different substitutes for antibiotics in the form of probiotics, prebiotics, symbiotics, phytobiotics, organic acids, as well as bacterial vaccines for the prevention of potential health problems. Such programs of application of alternatives to antibiotics have proven themselves well and, on some farms, antibiotics are used once or not at all per year. The level of applied biosecurity measures, which is a basic prerequisite for protection against pathogens, has a very huge influence on that. Farms with poorer biosecurity generally use more antibiotics. The data of our research show that by applying good biosecurity measures, and by using alternatives to antibiotics, antibiotics can be used more rationally and less often. Poultry farm production can be organized with minimal use of antibiotics with the application of all these measures.

Key words: antibiotics, rational use, poultry farms, biosecurity measures

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METABOLIC GENE EXPRESSION SIGNATURES IN RESPONSE TO RESIDUAL DOSES OF ANTIBIOTICS IN BROILER CHICKENS

Anna Slawinska[1*], Maria Siwek[2], Aleksandra Dunislawska[2], Sebastian Knaga[2], Malgorzata Olejnik[1]

[1]Nicolaus Copernicus University; Faculty of Biological and Veterinary Sciences, Toruń, Poland
[2]Bydgoszcz University of Science and Technology, Faculty of Animal Breeding and Biology, Bydgoszcz, Poland

* slawinska@umk.pl

Farm animals are main consumers of the antibiotics worldwide. Although in Europe the use of antimicrobials in chicken farming decreases, this trend is not applicable to Poland. The production of broiler chickens in Poland is still rising and currently Poland is the largest producer of the poultry meat in Europe, with nearly 3 million tonnes produced in 2022, over half of which is exported. Currently, the EU is implementing the regulations for the maximum cross-contamination of non-medical animal feed with antimicrobials, to reduce the unintended consumption by animals. Hereby we address this issue by analyzing the various effects of antibiotics residuals in the chicken feed at the levels allowed by the EU. The goal of this paper is to demonstrate how antibiotic residuals in the feed regulate the gene expression involved in metabolic processes in chicken liver and pancreas. The study was designed including six treatment groups and one control group. The treatment groups were fed diets containing residual doses of antibiotics (colistin, doxycycline, flumequine, thiamphenicol, tiamulin, and tilmicosin). The doses were calculated as 2% of the maximum approved concentration. The antibiotic-contaminated feed was delivered throughout the whole rearing period. The experiment was performed as follows: one-day-old female broiler chicks (line Ross 308) were randomly distributed to 42 pens (12 animals/pen, 504 animals in total). Each group included six pens.On days 7, 21, and 35 of rearing, 8 animals per group were slaughtered and dissected. The sampled tissues (liver and pancreas) were preserved in RNA stabilization solution. The RNA was isolated and RT-qPCR analyses were performed. The target gene panels included: *ACC*, *DGAT*, *FABP*, *FASN*, *LPL*, and *PPAR* (for liver), and *AMY2A*, *CCK*, *JAM2*, *OCCLUDIN*, and *PNLIP* (for pancreas). The reference genes were: *ACTB* and *GAPDH*. The relative gene expression was performed with ddCt method and the results were presented as Log_2 Fold Change (FC). The data were statistically analysed using non-parametric two-way ANOVA (SHR, Scheirer Ray Hare Test) with age and treatment group as independent variables (factors) and dCt values of target genes as dependent variables. The results indicate that age of the chickens was a significant variable for all target genes in liver and pancreas. The treatment group was significant for genes *FABP* ($p<0.001$) and *FASN* ($p<0.05$) in liver and *JAM2* ($p<0.05$) in pancreas. We found significant interactions between age and treatment for genes: *ACC*, *FASN*, and *LPL* ($p<0.001$), and *FABP* and *PPAR* ($p<0.05$) in liver, and *CCK* and *JAM2* ($p<0.05$) in pancreas. Overall, we found some distinct gene patterns in both tissues, regulated by age and antibiotic treatment. We conclude that even the residual doses of antibiotics allowed by law can trigger systemic changes in the broiler performance.

Key words: broilers, antibiotic residues, gene expression, metabolism

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PHARMACOKINETIC/PHARMACODYNAMIC ANALYSIS OF ENROFLOXACIN IN PORCINE SYNOVIAL FLUID IN GLAESSERELLA PARASUIS INFECTION

Zoltán Somogyi[1,2], Patrik Mag[1,2*], Ádám Kerek[1,2], Adrienn Mercédesz Veres[1,2]

[1]University of Veterinary Medicine Budapest, Department of Pharmacology and Toxicology, Budapest, Hungary
[2]National Laboratory of Infectious Animal Diseases, Antimicrobial Resistance, Veterinary Public Health and Food Chain Safety, University of Veterinary Medicine Budapest, Hungary

*mag.patrik@univet.hu

The study determined the main pharmacokinetic (PK) properties of enrofloxacin in porcine blood plasma and synovial fluid after a single subcutaneous administration of 7.5 mg/kg. The results were compared with pharmacodynamic (PD) results from the literature on *Glaesserella parasuis*. The experiment was performed using 10 DanBred genetics pigs, 10 weeks old, 29.51 ± 1.71 kg body weight. Blood and synovial fluid were collected from the animals at predetermined time points for 48 h. Drug concentrations were determined by LC-MS/MS. The main PK values (t_{max}, C_{max}, Cl, V_d, $AUC_{0-\infty}$, $t_{1/2}$, K_e) were calculated by non-compartmental pharmacokinetic analysis. Enrofloxacin reached its maximum concentration (C_{max}) in blood plasma in 5.03 ± 1.62 h (t_{max}), which was found to be 1.66 ± 0.33 μg/ml. In the synovial fluid, it reached a C_{max} of 1.67 ± 0.49 μg/ml in 8.60 ± 2.09 h. The clearance (Cl) of enrofloxacin in plasma was 0.19 ± 0.02 (l/h)/kg and in synovial fluid 0.18 ± 0.05 (l/h)/kg. The apparent distribution (V_d) was found to be 3.57 ± 0.64 l/kg in the former space and 2.96 ± 0.97 l/kg in the latter. The area under the curve ($AUC_{0-\infty}$) was 38.89 ± 3.89 (h × μg)/ml for blood plasma and 45.26 ± 12.68 (h × μg)/ml for synovial fluid. In the case of the pharmacodynamic target value (43.12 h) established in pigs infected with *G. parasuis*, the proposed PK/PD limit for septicaemia and arthritis caused by *G. parasuis* is 0.5 μg/ml with a probability of 90% (PTA).

Key words: enrofloxacin, synovial fluid, *Glaesserella parasuis*, PK/PD analyses

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BREEDING ROBUST PIGS FOR SUSTAINABLE PORK PRODUCTION - "CORRELATION BETWEEN ANTIBIOTIC USE AND PIG GENETICS"

Laszlo Buza[1,2*], Laszlo Gombos[3,4]

[1]University of Veterinary Medicine, Budapest, Hungary;
[2]Topigs Norsvin Central-Europe Ltd., Budaörs, Hungary; 3Széchenyi István University, Győr, Hungary; 4Agrofeed Ltd., Győr, Hungary;

*laszlo.buza@topigsnorsvin.com

It is essential that pigs exhibit enhanced robustness to disease to be successful in sustainable pork production systems. Breeding pigs for enhanced robustness to disease is possible, and can be facilitated using one of the following sets of genetic tools: 1.) traditional breeding, which makes use of existing genetic variation within a population; or 2.) genetic engineering, which can be used to introduce (novel) genetic variants. Topigs Norsvin's approach is to enhance overall robustness to disease, while placing emphasis on a few, specific diseases of interest (PRRS and Strep suis), using traditional breeding strategies. Evidence of substantial, naturally occurring genetic variation in robustness to disease can be found throughout the literature and has been detected both between and within Topigs Norsvin's genetic lines. An essential component of breeding pigs for enhanced robustness to disease using traditional breeding strategies is to maintain a reference dataset of animals with both disease phenotypes and genomic information. Together, these data can be used to estimate breeding values for robustness to disease. Topigs Norsvin's reference population consists of data collected following experimental inoculation with PRRS virus and natural exposure to various secondary pathogens, which captures differences in overall robustness to disease at the individual level. Topigs Norsvin recently completed a large-scale disease challenge trial in collaboration with a third-party research organization to evaluate the efficacy of this approach. This trial was designed as a proof-of-concept study to demonstrate that a reference dataset can be used to predict breeding values for robustness to challenge and furthermore, that these breeding values are predictive of robustness in the field. To address this objective, breeding values for robustness under disease challenge were estimated for all boars of the same genetic line within a single stud in the US. Next, boars were ranked based on these breeding values and the 30 top-ranking and 30 bottom-ranking boars were identified and used to sire the high robustness (HR) and low robustness (LR) groups, respectively. A total of 648 progeny per group were used for the trial. Pigs were placed in a commercial research barn at weaning and were vaccinated for PRRS using a modified live virus vaccine. Six weeks later, each pig was experimentally inoculated with the same infectious dose of PRRSV 1-7-4 and followed until marketing. Body weight and feed intake was recorded at the pen level. Additional weight measurements and mortality were recorded at the individual level. The most dramatic differences between groups were observed during the initial challenge phase (from 0 to 21 days post-infection), where the HR group had numerically greater gain and feed intake, as well as superior FCR. Differences between groups in mortality rate were most noticeable during this initial challenge period, where 100% more mortalities were observed for the LR group (n = 21) than the HR group (n = 11). Likewise, the HR group also had lower overall mortality rate, fewer culls, and more full value pigs. A statistically significant difference was detected between groups for antibiotic treatment, where 14% of pigs in the LR group required one or more antibiotic treatments throughout the duration of the study, vs. only 10% of pigs within the HR group. In conclusion, results from this study provide evidence that traditional breeding is an effective approach to breeding pigs for enhanced robustness to disease. Results from this proof-of-concept study show that selecting boars based on high vs. low experimental breeding values for robustness resulted in robustness differences between their progeny groups. Pigs sired by boars with high experimental breeding values for robustness had numerically better growth performance, fewer mortalities, more full-value pigs, and

required significantly fewer antibiotic treatments than pigs sired by boars with low experimental breeding values. Further, twice as many pigs from the LR group died within the first 3 weeks post PRRS-challenge, compared to pigs within the HR group.

Key words: pig, genetic, breeding, sustainability, antibiotic use, robustness, PRRS

ANTIMICROBIAL RESISTANCE SURVEILLANCE IN REPUBLIC OF NORTH MACEDONIA WITH FOCUS ON CARBAPENEM RESISTANT ENTEROBACTERIA AS AN ACTUAL PROBLEM IN CLINICAL PRACTICE

Ana Kaftandzieva[1], Biljana Kakaraskoska-Boceska[2]

[1]Institute of Microbiology and Parasitology, Faculty of Medicine, UKIM, Skopje, President of the Multisectoral Commission for Control of Antimicrobial Resistance
[2]National CAESAR data manager, Institute for Public Health, Skopje, Republic of North Macedonia

*akaftandzieva@yahoo.com

Antimicrobial resistance (AMR) is one of the three biggest problems of modern medicine. It is estimated thatAMR will surpass cancer and cardiovascular diseases as the leading cause of death, by 2050. The aim of this study is to make an overview on the current and future activities in the field of AMR and present CAESAR results for the past 5 years. The AMR multi-sectoral commission was established in 2009 andsince 2013, activities have been held every year to mark WAAW and EAAD. Macedonia has been part of the CAESAR network since the beginning in 2013. The number of submitted invasive isolates for the last 5 years has increased from 266 isolates in 2018 to 369 isolates in 2022. *K. pneumoniae* and *S. aureus* are the most common invasive isolates through the years, although the number of *Acinetobacter baumannii* is constantly raising. We are dedicated to promote new and enhance already existing methods for AMR detection as well as surveillance software like WHONET. Various projects with ECDC, WHO and EC in this field are ongoing, such as EuroGenRefLabCap, ENLabCap, TrACSS, ECDC-AMRWB - Country Support to Advance One-Health Responses against Antimicrobial Resistance in Western Balkans, etc. Close collaboration with the National Health Insurance Fund has been established in order to reduce the usage of antimicrobials and by prescriptiononly. Continuous education among medical students, GPs and relevant specialties will play a crucial role in the future activities. Establishing referent AMR laboratories in the country is our priority goal as a next step. Dedicated and focused work in AMR surveillance is more than needed forthe Balkan region. The government of RNM, the WHO office in Skopje, the IPH of RNM, will continue their activities to establish good collaboration between countries. Considering enterobacteria, the most common carbapenem resistant species is *Klebsiella pneumoniae*. During a five-year-period (2017-2021) all samples (urine, wound, blood, tubus/canila, sputum, tracheal aspirates) obtained from the hospitalized patients were analyzed at the Institute of Microbiology and Parasitology, Faculty of Medicine, Skopje. Determination of the susceptibility was done by disk diffusion method, Vitek 2 and E-tests. CIM method and carbapenemase set (Mast Diagnostic) were performed for detection of carbapenemase production to all strains that showed smaller inhibition zones to carbapenems according EUCAST. Easyplex SuperBug CRE (Amplex Biosystems, Germany) was performed for detection of beta-lactamase genes. Out of the total number of *Klebsiella pneumoniae*–Kp, carbapenem resistance was detected in 18-22% of the strains. In 90 randomly selected isolates with positive phenotypic tests for carbapenemase detection, metallo-beta lactamases encoding genes were detected, such as: NDM (89.5%), OXA-48 (9.2%), VIM (1.3%). Between 90-100% of Kp strains were resistant to 9 tested beta-lactams and ciprofloxacin. The resistance to meropenem and to cotrimoxazole was between 80-89%, to imipenem 50-80%, to gentamicin 40-80% and to amikacin 30-50%. Increased MICs to colistin were detected in 12,5-18% of all isolates in a tested period. The percentage of CRKp isolates is increasing and it is the right time to perform all strategies to control their spread.

Key words: antimicrobial resistance, CAESAR, Carbapenem-resistance, *Klebsiella pneumoniae,* antibiotics

LACTONASE MEDIATED QUORUM QUENCHING OF *PSEUDOMONAS AERUGINOSA* VIRULENCE

Milka Malešević[1*], Jovana Ćurčić[1], Branko Jovčić[2]

[1]Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia
[2]Faculty of Biology, University of Belgrade, Belgrade, Serbia

*milkam@imgge.bg.ac.rs

Solving the problem of the antimicrobial resistance crisis is one of the primary challenges currently confronting the healthcare system. One of the most promising new strategies to combat antimicrobial resistance is the antivirulence therapy, based on silencing bacterial cell-to-cell communication (quorum quenching - QQ). QQ enzymes lactonases represent a diverse group of enzymes capable of inactivating signaling molecules of bacterial communication – N-acyl homoserine lactones (AHLs), resulting in alterations ofbacterial virulence. The numerous virulence factors and resistance to most conventional antibiotics have led to *Pseudomonas aeruginosa* being listed as one of the top-priority pathogens on the ESCAPE pathogen list, highlighting the urgent need for the development of new therapies to combat this pathogen. *P. aeruginosa*uses cell-to-cell communication known as quorum sensing (QS) that allows bacteria to monitor their own population density via signal molecules and subsequently control bacterial pathogenesis. Our hypothesis was that bacterial pathogens which share the same ecological niche with *P. aeruginosa* during infection have developed a system to disrupt its QS system, in order to survive in polymicrobial communities alongside this successful pathogen. In our research we identified QQ enzymes lactonases originating from two Gram-negative bacterial pathogens *Burkholderiacepacia* and *Stenotrophomonas maltophilia*. The genes encoding for the enzymes were cloned and expressed in pQE30 expression vector. *B. cepacia* BCC4135 synthesizes two lactonases YtnP and Y2-aiiA, that have the different cellular localization, but also different substrate specificity, which could imply the difference in their biological roles. *S. maltophilia* 6960 YtnP lactonase has several advantageous biotechnological properties, such as high thermostability, activity in a wide pH range, and no cytotoxic effect. Fluorescence microscopy analysis showed a strong effect of analyzed lactonases on preventing biofilm formationand initiating the decomposition of the preformed biofilm of *P. aeruginosa* MMA83. Functional assays showed that lactonases have the ability to significantly reduce extracellular virulence factors production – elastase, pyocyanin and rhamnolipid. Additionally, the results obtained by real-time quantitative PCR showed that analyzed recombinant enzymes significantly downregulated all three analyzed *P. aeruginosa* QS networks at the transcriptional level. Finally, *S. maltophilia* 6960 YtnP lactonase significantly prolonged survival of *Caenorhabditis elegans* by reducing virulence of *P. aeruginosa* using fast-killing liquid assay. The described properties make *B. cepacia* and *S. maltophilia* lactonases the promising therapeutic candidates for the development of next-generation antivirulence agents.

Key words: quorum sensing, quorum quenching, lactonase, *Pseudomonas aeruginosa*, biofilm, virulence, antivirulence therapy

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THE IMPACT OF ANTIBIOTIC INTERACTIONS ON THE PATHOGENS GENERATING RESISTANT HOSPITAL INFECTIONS

Bojana Božić Cvijan[1*], Milica Bajčetić[1,2]

[1]Department of Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Belgrade, P.O. Box 38, 11000 Belgrade, Serbia
[2]Clinical Pharmacology Unit, University Children's Hospital, 11000 Belgrade, Serbia

*bojanabozic87@gmail.com

Bacterial resistance is a global health problem that is occurring at higher speed compared to antibiotic development. Therefore, the role of already existing antibiotics in combinations need to be fully addressed. Central Asian and European Surveillance of Antimicrobial Resistance (CAESAR) network marked *Escherichia coli* (*E. coli*), *Klebsiella pneumonia* (*K. pneumonia*), *Salmonella* species (spp.), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Acinetobacter* spp., *Staphylococcus aureus* (*S. aureus*), *Streptococcus pneumonia* (*S. pneumonia*), *Enterococcus faecalis* (*E. faecalis*), *Enterococcus faecium* (*E. faecium*) as pathogens of great importance due to their potential to cause invasive infections and to spread in the community or health settings, and limited therapeutic options. Having in mind high resistance rate of nine mentioned pathogens, multidrug antibiotic combinations are often used. Studies that showed increased effect after antibiotic combination have great impact in clinical settings and may provide a beneficial alternative for the treatment of these multidrug resistant bacteria. Contrary, several antibiotic combinations provided no benefit or similar effects regarding clinical or antimicrobial effect to comparator, which raises concern about polypharmacy and antibiotic stewardship. Results of decreased effect after antibiotic combination provide important knowledge how to prevent development of bacterial resistance. Focus on new large randomized controlled studies that will investigate the impact of antibiotic combinations should be adjusted to the needs of national and international surveillance systems.

Key words: antibiotic, interactions, combination, resistance, antagonism

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THE ROLE OF CLINICAL PHARMACOLOGIST IN PROMOTING RATIONAL ANTIBIOTIC USE – EXPERIENCE FROM UNIVERSITY HOSPITAL CENTRE OSIJEK, CROATIA

Suzana Mimica[1,2*]

[1]Unit for Clinical Pharmacology, Department of Internal Medicine, University Hospital Centre Osijek, Croatia
[2]Department of Pharmacology, Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Croatia

*suzana.mimica@kbco.hr, suzanamimica24@gmail.com

Specialists of clinical pharmacology are doctors of medicine that in Croatia undergoe 4 years of specialty training and are employed in hospital setting (often also in academia). Their focus of professional interest is promoting rational drug use in all aspects of pharmacotherapy, including the use antimicrobial agents. In University Hospital Centre (UHC) Osijek clinical pharmacologists are involved in antimicrobial stewardship programme and are members of Committe for antibiotics use and Committee for intrahospital infections. They are involved in antibiotics use policy at the hospital level, in creating the list of reserve antibiotics and approval of those antibiotics. They are part of interdisciplinary expert team that consults antibiotics use in Intensive Care Units. Every year we quantitatively analyze antibiotcs use according to DDDs per 100 bed-days and send that report to the central place in Croatia where they are collected and sent to European Centre for Disease Prevention and Control (ECDC). At the national level, clinical pharmacologists were part of the expert groups that prepared and wrote the national guidelines for some conditions (e.g. Urinary tract infections, Bacterial tonsillopharyngitis, Prostatitis etc). Clinical Pharmacologists from University hospital centresin Rijeka and Zagreb, in collaboration with microbiologists and other specialist have published The guidelines for hospital antimicrobial drug use (with several updated versions) but also The guidelines for the antibiotic use in primary care, for general practiotioners. One of the important tasks of clinical pharmacologists is evaluating and testing *in vivo* hypersensitivity to all types of antimicrobials. As approximately 10% of all population is labelled with penicillin allergy and afterwards only in 5-10% of them the allergy is confirmed, it is important to de-label penicillin allergy in patients without it. This enables the use of narrow spectrum antibiotics (e.g. penicillin G or V) and prevents collateral damage seen with the use of wide spectrum antibiotics.Some current important problems in hospital antibiotics use are: prolonged use of antibiotics given as perioperative prophylaxis, too long antibiotics use in general, high fluoroquinolones consumption, non-rational combinations of antibiotics, lack of de-escalation procedures, lack of software support before initiation of antibiotic use (the choice of the agent, possible drug-drug interactions, dose adjustment to renal function).

Key words: hospital drug use; antibiotics consumption; rational drug use; antibiotic guidelines

ASSESSMENT OF SEASONAL AIRBORNE RESISTOME DYNAMICS IN RESPONSE TO AIR POLLUTION EXPOSURE IN THE BELGRADE METRPOLITAN AREA

Katarina Novović[1*], Danka Matijašević[1], Milka Malešević[1], Lazar Gardijan[1], Stefan Stanovčić[1]

[1]Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia

*katarinanovovic@imgge.bg.ac.rs

Antimicrobial resistance (AMR) and air pollution have been identified as one of the most serious threats to human health worldwide. The scarce data demonstrating their interdependence indicates a need to obtain evidence from a broader global area, especially from regions exposed to high air pollution. Considering that Serbia is a country struggling with excessive antibiotics use and misuse, a high percentage of multidrug-resistant bacterial isolates, and poor air quality, the Serbian capital Belgrade has been recognized as an interesting research model for the effects of air pollution on the airborne transmission of AMR. Hence, this abstract aims to present the concept of an ongoing project (AirPollRes) that points to the additional risk dimension of air pollution to human, animal, and environmental health. After optimization of air sampling and DNA extraction protocol, the air samples will be collected at nine locations in the Belgrade metropolitan area selected according to air pollution level during four seasons. The state-of-the-art shotgun metagenomic sequencing and bioinformatic analysis of obtained sequences will provide information about microbial community composition of airborne metagenomes. In addition, sequenced airborne metagenomes will be analyzed for the abundance and diversity of resistomes (antibiotic and biocide/metal resistance genes) and mobilomes using several databases and tools. Correlation analyses will offer us insight into the effect of air pollution and seasonal variations on abundance and diversity of airborne pathogens, resistome and mobilome in the Belgrade metropolitan area. In-depth approach of the AirPollRes project will provide the first insights into intersection of AMR and air pollution in the Belgrade metropolitan area, which is highly vulnerable to these health threats. As the AirPollRes is a pioneering project in this field, the expected short-term impact is the introduction of routine monitoring of pathogenic microbes and resistance determinants in the air in Belgrade, while the longer-term impact will be reflected in the improvement of human and animal health, allowing for a longer life with higher quality.

Key words: antimicrobial resistance, air pollution, airborne metagenome, resistome, mobilome, Belgrade

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CURRENT STATUS OF MULTIDRUG RESISTANT ORGANISMS IN CRITICALLY ILL CHILDREN-A GROWING CONCERN: REPORT FROM THE INSTITUTE FOR CHILD AND YOUTH HEALTH CARE IN VOJVODINA, SERBIA

Izabella Fabri Galambos[1*]

[1]Institute for Child and Youth Health Care Vojvodina, Faculty of Medicine, University of Novi Sad, Serbia

*izabellafabri@gmail.com

Infections acquired during hospitalization, also known as nosocomial or intrahospital infections, pose a significant challenge in healthcare settings worldwide. These infections can result from various factors such as invasive procedures, compromised immune systems, prolonged hospital stays, and microbial resistance to antibiotics. Understanding the types of intrahospital infections, their causative agents, and factors contributing to their development is crucial for effective prevention and management. The causative agents of intrahospital infections in children vary based on factors such as the patient's underlying disease, exposure to invasive procedures, non-invasive procedures, and the affected part of the body. Children, especially those under two years of age, are more susceptible to intrahospital infections. Common infections in pediatric patients include hematological infections, respiratory tract infections, and urinary tract infections. One of the major challenges in treating intrahospital infections is the emergence of microbial resistance to antimicrobial drugs. The profile of antimicrobial resistance among pathogens changes annually, necessitating regular monitoring of local and regional epidemiological data to guide effective treatment strategies. Addressing intrahospital infections requires a multidisciplinary approach involving healthcare providers, infection control specialists, microbiologists, pharmacists, and hospital administrators. Collaboration and communication among these stakeholders are essential for implementing comprehensive infection prevention and control strategies. In the Institute for child and youth healthcare in Vojvodina, Serbia surveillance of resistance to antibiotic agents is continuously followed from 2019. Years of monitoring shows a progressive growth of resistance to antibiotic agents with an alarming speed.

Key words: children, intrahospital infections, bacterial resistance

PHARMACOECONOMICS AND PHARMACOEPIDEMIOLOGY OF ANTIMICROBIAL THERAPIES

Jovan Mihajlović[1,2,3*]

[1]Mihajlović Health Analytics (MiHA), Novi Sad, Serbia
[2]University Medical Center Groningen, Groningen, The Netherlands
[3]Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia

*jovan.mihajlovic.rs@gmail.com

Pharmacoeconomics is a scientific discipline focussed on the economic evaluation of medicines. It assesses differences in costs and effectiveness/utilities of various medical treatment to assist decision-makers, such as healthcare providers, insurers, and policymakers, in rational resource use in healthcare. Pharmacoeconomic analysesaim at efficient allocation of the health care budget and maximization of health gains within such a budget. These evaluationscan consider only direct costs of treatments (all costs related to the treatment of the disease), and therefore take "a healthcare payer perspective", or can consider both direct and indirect costs (related to the absence of a patient and informal care giver from the economic contribution to the society), and thus take "a societal perspective". Key types of pharmacoeconomic analyses include: 1) Cost-minimization analysis (CMA) which compares medicines of identical effectiveness (same/generic INNs); 2) Cost-effectiveness analysis (CEA) which analyses the costs of treatments within a same therapeutic group relative to its outcomes which are specific for that therapeutic group; 3) Cost-utility analysis (CUA) which compares costs relative to the quality-adjusted life years (QALYs) gained; 4) Net-monetary benefit (NMB) analysis which compares the costs of a treatment with its monetary benefits. Pharmacoepidemiology examines the use, effects, and outcomes of medications in large populations. When applied to antibiotics, pharmacoepidemiology focuses on studying the patterns of antibiotic use, the factors influencing prescribing practices, the emergence of antimicrobial resistance, and the impact of antibiotic use on public health. Pharmacoepidemiological studies investigate the prevalence and trends of antibiotic prescribing in various healthcare settings, such as hospitals, clinics, and community pharmacies. These studies analyze factors influencing prescribing decisions, including patient demographics, clinical characteristics, healthcare provider behavior, and healthcare system factors. Pharmacoepidemiology plays a crucial role in monitoring the emergence and spread of antimicrobial resistance (AMR). By studying antibiotic usage patterns and resistance trends, researchers can identify associations between antibiotic use and the development of resistant bacteria. This information helps inform antimicrobial stewardship programs and public health interventions aimed at combating AMR. Understanding the pharmacoepidemiology of antibiotics is essential for evaluating the impact of antibiotic use on public health outcomes, such as healthcare-associated infections, antibiotic-resistant infections, healthcare costs, and patient outcomes. By quantifying the burden of antibiotic-related issues, policymakers can develop strategies to optimize antibiotic use and mitigate the risks associated with antimicrobial resistance. Overall, pharmacoepidemiological research on antibiotics plays a critical role in promoting the rational use of antibiotics, minimizing the development of antimicrobial resistance, and ensuring the effectiveness of antibiotic therapy for future generations.

Key words: antimicrobials, antimicrobial resistance, pharmacoeconomics, pharmacoepidemiology, therapy

DO WE NEED NEW DRUGS IN THERAPY OF *OTITIS EXTERNA*, OR THE OLD ONES STILL WORKS?

Natalija Milčić Matić[1*], Isidora Prošić[1]

[1]Faculty of Veterinary Medicine, University of Belgrade, Serbia

*natalija.milcic@vet.bg.ac.rs

Canine otitis externa is a common dermatologic problem in small animal practice. Affected ears are often painful and pruritic often with an unpleasant-smelling discharge. Canine otitis externa, prevalent in 5% to 20% of dogs globally, is a common reason for antibiotic prescriptions in small animal practice and raises concerns as dogs may serve as potential reservoirs of antimicrobial resistance (AMR) determinants, impacting public health. This study aimed to determine the prevalence of various bacteria causing clinical ear infections in dogs and describe the AMR patterns of the primary causative agents of canine otitis. A total of 167 ear swabs were collected from patients at the Dermatology Clinic at the Faculty of Veterinary Medicine in Belgrade, Serbia, between 2017 and 2023. 218 bacterial strains were isolated, with mixed infections detected in 50 (29.94%) samples. Additionally, 218 antibiograms were analyzed. From the 167 ear swabs, the most prevalent isolated species was *Staphylococcus pseudintermedius*, found in 72 ear swabs (43.11%), followed by *Pseudomonas aeruginosa*, isolated from 48 ear swabs (28.74%). *Proteus* spp. accounted for 15.57%, *Streptococcus* spp. for 12.57%, *S. aureus* and coagulase-negative staphylococci for 9.58% each, and Enterobacteriales (excluding *Proteus* spp.) for 4.79%. A minimum of 12 antibiotic compounds from 9 antibiotic classes were tested for each strain. Depending on the type of bacteria tested, resistance ranged from 12.5% to 93.75% for penicillins, 12.5% to 93.75% for cephalosporins, 12.5% to 52.77% for fluoroquinolones, 3.85% to 76.67% for aminoglycosides, 27.27% to 75% for macrolides, 0% to 78.72% for amphenicols, 22.22% to 95.5% for sulfonamides, 43.75% to 97.5% for tetracyclines, and 6.25% to 40% for imipenem. Effective treatment of ear infection includes treatment of infection and inflammatory changes as well as determination of the underlying factors that led to development of otitis in the first place. Topical therapy is the mainstay treatment for otitis externa although systemic use of anti-inflammatory therapy and/or antimicrobial therapy may be indicated for individual patients. Many commercially available topical ear medications contain a combination of antibiotics, antifungals, and glucocorticoids and are supplied in different formulations for daily or weekly applications. Common antibiotics are aminoglycosides (gentamicin, neomycin), fluoroquinolones (ciprofloxacin, enrofloxacin, marbofloxacin), polymyxin B, fusidic acid and florfenicol. Considering the small number of topical antibiotics available, and the relatively high resistance of bacteria diagnosed in the ear canal, in some cases we have to apply antisepticsor injectable antibiotics in different concentrations. Continued surveillance is vital to grasp antimicrobial resistance trends in veterinary medicine. This study underscores the necessity of establishing an antimicrobial resistance surveillance program in small animal practice, especially with the rising number of pet owners. Regular monitoring is crucial for understanding and addressing antimicrobial resistance in companion animals, particularly in Serbia, where surveillance programs are lacking despite global recommendations.

Key words: otitis externa, dogs, antimicrobial resistance, antibiotics

PHAGE THERAPY FOR SKIN DYSBIOSIS IN DOGS

Tina Kotnik[1*], Iva Šumonja[2]

[1]Small Animal Clinic, Veterinary faculty, University of Ljubljana, 1000 Ljubljana, Slovenia
[2]Veterinary practice Uvodić, Mavrinci 2, 51219 Čavle, Croatia

*tina.kotnik@vf.uni-lj.si

Many skin diseases in humans and animals are associated with an imbalance in the skin microbiome, recently termed dysbiosis. In microbial dysbiosis, diversity is reduced and certain species are more prevalent. Antimicrobial therapy restores the diversity of the skin microbiome; however, this effect can diminish after treatment is discontinued. Therapies for skin dysbiosis have traditionally included antibiotics and antiseptic medications. Due to increasing microbial resistance to antibiotics, research into alternative treatment options has been driven forward. Phage therapy can be considered the third important intervention for the treatment of bacterial infections after vaccines and antibiotics. Bacteriophages are viruses that can infect and kill bacteria without having negative effects on human or animal cells. Research results on phage therapy for skin dysbiosis in dogs are collected. The clear clinical potential of phage therapy, its advantages and disadvantages, and some legal, biological and technical challenges it faces for its further implementation and wider application will be outlined.

Key words: dysbiosis, bacterial overgrowth, bacteriophages, phage therapy

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ANTIBIOTIC SENSITIVITY OF BACTERIAL ISOLATES FROM CANINE EJACULATES

Jelena Apić[1*], Stevan Rodić[1], Tomislav Barna[1], Ivan Galić[2], Ivan Stančić[2], Igor Stojanov[1], Zorana Kovačević[2]

[1]Scientific Veterinary Institute "Novi Sad", Novi Sad, Republicof Serbia
[2]University of Novi Sad, Faculty of Agriculture, Department for Veterinary Medicine, Novi Sad, Serbia

*jelena.a@niv.ns.ac.rs

Dog breeders or owners of dogs of different breeds and ages, at the request of the practicing veterinarian or at their own request, submit ejaculate samples for bacteriological analysis. The health and hygiene checking of the ejaculate immediately before the planned natural mating or artificial insemination of bitches should be a regular preventive activity. However, most often breeders/owners turn to veterinary services when they notice that after several matings, the females do not become pregnant. In the period from January 2021 to March 2024, a total of 38 ejaculates from males with an average age of 3.8 years (from 1 to 11 years) were examined in the laboratories of the Scientific Veterinary Institute "Novi Sad", Republic of Serbia. The dogs were of the following breeds: Bernese Mountain Dog, Staffordshire Bull Terrier, Central Asian Shepherd, Yorkshire Terrier, German Hunting Terrier, Pomeranian Spitz, German Shepherd, Caucasian Shepherd, Golden Retriever, Cane Corso and Doberman. The aim of this study was to examine the presence of pathogenic microflora in dog ejaculate samples, as well as the sensitivity of those isolates to antimicrobial drugs. Through bacteriological analysis of a total of 38 examined samples, pathogenic bacteria were isolated in 17 ejaculate samples (44.74%). The following bacteria were isolated: *Escherichia coli* (n=4/17; 23.53%), *Staphylococcus pseudintermedius* (n=10/17; 58.82%), *Streptococcus canis* (n=3/17; 17.65%), *Streptococcus viridans* (n=2/17; 11.76%), *Proteus* sp. (n=1/17; 5.88%). Based on the obtained results, we conclude that different isolates of the same bacterial strain react very differently to the same antimicrobial drug.

Key words: antibiotics, antibiotic resistance, bacteria, dogs, sperm.

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EFFECT OF ANTIMICROBIALS AND PROBIOTICS ON GUT MICROBIOTA IN COMPANION ANIMALS

Nataša Golić[1,2*], Amarela Terzić-Vidojević[1], Katarina Veljović[1], Nikola Popović[1], Igor Mrvaljević[2], Damjan Damnjanović[2], Andrej Kravčuk[2]

[1]Institute of Molecular Genetics and Genetic Engineering University of Belgrade, Belgrade, Serbia
[2]Invetlab DOO Beograd, Belgrade, Serbia

*natasag@imgge.bg.ac.rs

Early gut microbiota colonization immediately after birth has a significant impact on the body's functioning, necessary for immunity development and good healthmaintaining. The interaction of gut microbiota and the host is vital for animals, since it is crucial in food digestion processes, protection against various diseases, and the functioning of distant organs including the central nervous system. However, various external factors (nutrition, infection, stress, inflammation, antibiotics) can lead to disturbances in the gut microbiota composition and diversity, so-called dysbiosis. Hence, the understanding of gut-microbiota cross-talk is highly needed. In companion animals (dogs and cats), belonging to the order Carnivora, the diet is traditionally based on food with a high protein and fat content with a relatively low concentration of fiber. Although the digestive tract of dogs and cats is relatively simple, their microbiota is rich and diverse. However, the process of "humanization of pets" has led to the development of complex diseases, such as obesity, diabetes, metabolic syndromes, inflammatory bowel diseases, cancer, and mood disorders. All these diseases are related to diet and gut microbiota dysbiosis. Unfortunately, the problems of gut microbiota dysbiosis are still most often solved by the use of antibiotics, leading to additional gut microbiota disruption and to a final extent the development of antimicrobial resistance, which represents a huge global problem today. The use of different 'omics' methods has enabled a new era of studying the role of gut microbiota in animal health and disease, especially in terms of host-microbiota interactions. New findings have highlighted the potential of new therapeutic approaches based on gut microbiota modulation to improve animal health. To reduce the use of antimicrobial agents and the spread of antimicrobial resistance among commensal and pathogenic bacteria the use of probiotics is highly recommended. Probiotics are defined as "live microorganisms, which when administered in adequate amounts have a beneficial effect on the health of the host." Probiotic characteristics depend on the strain and can be realized through several mechanisms: i) production of antimicrobial compounds; ii) competitive binding to intestinal epithelial cells blocking the receptors for binding of pathogens; iii) strengthening the tight junctions of the intestinal epithelium; iv) induction of intestinal mucosa production; and c) bursting the innate immune response. We have developed an innovative probiotic as an alternative to the use of antimicrobial agents in animals – HiraPet. HiraPet consists of three strains of lactic acid bacteria: *Streptococcus thermophilus* BGVLJ1-44, *Lactobacillus fermentum* BGHI14, and *Lactobacillus helveticus* BGRA43. The strain *Lactobacillus helveticus* BGRA43 exhibits a strong antimicrobial effect against enteropathogenic bacteria *Yersinia enterocolitica*, *Shigellasonnei*, *Shigellaflexneri* (London 9950), *Streptococcus pneumonia*, *Clostridium difficile*, *Clostridium perfringens* and enterotoxic *Escherichia coli* (ETEC). HiraPet probiotic exhibits protective immunomodulatory activity, contributing to maintaining of the intestinal epithelial barrier and positively affecting the diversity of gut microbiota.

Key words: gut microbiota, probiotics for companion animals, alternative to antimicrobial agents, *Streptococcus thermophilus* BGVLJ1-44, *Lactobacillus fermentum* BGHI14, *Lactobacillus helveticus* BGRA43

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HUMAN APPROVED ANTIMICROBIALS IN THE SMALL ANIMAL PRACTICE: WHEN AND WHY?

Zorana Kovačević[1*], Dragana Tomanić[1]

[1]Department of Veterinary Medicine, Faculty of Agriculture, University of Novi Sad, Serbia

*zorana.kovacevic@polj.edu.rs

Excessive and inappropriate utilization of antimicrobials (ABs) significantly drives the development of antimicrobial resistance (AMR) in the humans, animals, and their surrounding environments. Small animals are frequently treated with ABs and could be considered as reservoirs of bacteria resistant to ABs thereby presenting a potential risk for zoonotic transmission as they live near humans. Assessing ABs prescription practices and promotion of responsible ABs use are crucial aspects of antimicrobial stewardship (AMS) programs. However, the utilization patterns of ABs in the small animals are reported less frequently compared to those in food-producing ones. Drugs approved for use in humans are frequently used for treating infections in the small animals, such as dogs and cats, known as "off label" use. Moreover, small animals share some classes of ABs, including those that are medically important, promoting the emergence of AMR relevant to human health. Consequently, addressing the misuse of AB, especially those categorized as the highest priority critically importance (HPCIAs), in the small animals is progressively gaining significance within the One Health approach. The World Health Organization (WHO) classified antimicrobials into Access, Watch, and Reserve groups (AWaRe classification) to promote prioritization, and surveillance of the HPCIAs and support AMS efforts at local, national, and global levels. Hence, the aim of the present study was to assess the small animal veterinarians prescribing practice regarding human-approved ABs in dogs and cats. Furthermore, focus will be on the evaluation of these ABs based on WHO critically important ones in human medicine and AWaRe characterization. This questionnaire-based study was conducted among licensed veterinary practitioners employed at the veterinary clinics within the territory of Južna Bačka district, Serbia for 4 months, (from beginning of January to end of April of 2024) using the Google Forms platform. The questionnaire was filled in by 96 licensed veterinary practitioners involved in the small animal practice representing response rate of 28%. For canine prescriptions, the results indicate that beta lactams were the most frequently prescribed human-approved ABs (48.4%), followed by nitroimidazoles (18%) and tetracycline (11%). Among beta lactams, HPCIAs are cefixime (13%) and cefpodoxime (2%). Besides, quinolones are HPCIAs prescribed in 1% of cases. Additionally, a significant proportion of prescribed ABs in dogs fall under the highly important category (HIA), such as amoxicilin/clavulanic acid (52%), followed by metronidazole (18%) and doxycycline (11%). These two ABs (metronidazole and doxycycline) are both in the Access group due to AWaRe classification. The most frequently prescribed human-approved ABs for the treatment of cats were beta lactams (60%), followed by macrolides-lincosamides (13%). Only prescribed HPCIAs was cefixime, presented in the 14% cases of all beta lactams. The most frequently prescribed ABs that are HIA and in the Access group were metronidazole (18%) and clindamycin (7%).These results underscore the substantial use of ABs crucial in human medicine for managing canine and feline infections, thus emphasizing the close relationship between human and veterinary medicine in addressing infectious diseases. In conclusion, following the cascade procedure principles, veterinary practitioners should prescribe human approved ABs only if a suitable veterinary drug for another animal species does not exist. Through targeted interventions informed by data on prescribing practices, it is important to work towards optimizing ABs use in the small animals, safeguarding their health, and preserving the effectiveness of these crucial medications for future generations.

Key words: antimicrobial resistance, antimicrobial stewardship, off label, One Health approach, small animals

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ANTIFUNGAL-RESISTANCE FROM BOTH HUMAN HEALTH AND PLANT PROTECTION PERSPECTIVES

Li-Jun Ma[1*]

[1]Department of Biochemistry and Molecular Biology, College of Natural Sciences, University of Massachusetts Amherts, United States of America

*lijun@umass.edu

Fusarium oxysporum is a cross-kingdom fungal pathogen that not only causes devastating plant vascular diseases, but can also opportunistically infect humans. *Fusarium* spp. are refractory to therapy and exhibit broad resistance to most clinically available antifungals. Environmental changes such as contamination with fungicides have also created pressures for microorganisms to adapt rapidly, promoting the creation of new emerging fungal diseases for animals and humans. This study compared genome dynamic of a plant pathogenic strain *F. oxysporum* f. sp. *lycopersici* 4287 (Fol4287) and an eye infecting strain MRL8996 under short-term experimental evolution to study how these two strains adapt to different environment stresses. Serial passaging of a clonal isolate resulted in populations displaying significantly increased fitness in the respective passaging condition. Sequencing of the evolved lines revealed changes both at the nucleotide and chromosome level, some are fixed in the population. Strikingly, most detected variants in both evolving passages were transposable element insertions. However, the DNA transposons (mainly hAT and Tc1-Mariner superfamilies) are highly active in the plant pathogen Fol4287, while retrotransposons (specifically SINEs) are active in the eye infecting strain MRL8996. In Fol4287, TE insertions show a bias for sequences with H3K27me3, whereas in MRL8996 TE insertions are disproportionately inserted in upstream and downstream regions of genes. Interestingly, multiple independent lineages evolved under the same conditions carry events at the same locus, providing bases to investigate rapid evolution instances that may result in environmental adaptation. Repeated mutations are observed for *VeA* and *VelB*, the core members of the Velvet complex that affect a number of functions important in reproduction, production of secondary metabolites and host-microbe interactions. Collectively, our results suggest that transposable elements are major short-term evolutionary forces in *F. oxysporum*, providing new insights into the genetic mechanisms underlying host adaptation in this important fungal pathogen.

Key words: antifungal resistance, *Fusarium oxysporum*, genome dynamics, genetic mechanisms

ENVIRONMENTAL IMPACT OF ANTIBIOTIC USE IN PLANT PROTECTION

Milan Ivanović[1*], Anđelka Prokić[1], Jelena Adamović[1], Aleksa Obradović[1]

[1]University of Belgrade, Faculty of Agriculture, Belgrade, Serbia

*milanivanovic@agrif.bg.ac.rs

Plant pathogenic bacteria (PPB) cause devastating crop losses worldwide. In order to reduce their impact, antibiotics have been used for management of bacterial diseases since 1950s. Their application ismostly restricted to a high value fruit, vegetable, and ornamental plants. The most common target is *Erwinia amylovora*, the causal agent of fire blight, which affects apple, pear and quince, infecting flowers, leaves and shoots. Antibiotics are usually applied preventively 1-2 times during the flowering stage, while they are considered ineffective after infection occurs. Soon after the introduction of antibiotics into plant protection programs, bacterial strains resistant to streptomycin emerged. Although the diversity and total amounts of antibiotics used in plant protection are muchlower compared to use in animal farming, their applicationon plants is the subject of debate. It has been hypothesized that spraying antibiotics into open environmental spaces might increase the frequency of antibiotic resistance genes (ARGs) in bacteria living on plant surfaces which could then be transferred through horizontal gene transfer into clinically significant bacteria. In three independent surveys we tested over 100 strains of *Xanthomonas euvesicatoria*, 40 strains of *Erwinia amylovora* and 46 strains of *Xanthomonas arboricola* pv. *corylina*, collected from different locations and years, for resistance towards streptomycin, kasugamycin and copper sulfate. All tested strains were sensitive to streptomycin, predominantly sensitive to kasugamycin (some strains showing resistance to 50 or 100 ppm of kasugamycin) and resistant to copper sulfate. While all strains tolerated 100 ppm of copper sulfate, 41 out of 141 showed resistance at 200 ppm of this bactericide. These studies suggested possible copper resistance development in some bacterial populations in Serbia, due to intensive use of this compound in PPB control. Conversely, reduced sensitivity to kasugamycin is presumably an inherited trait since there is no official evidence on the use of kasugamycin in plant protection in Serbia. Legislation in European countries, including Serbia, does not authorize antibiotics as plant protection products (PPPs). However, due to inadequate control over antibiotic sales and import in Serbia, antibiotics are applied to some extent, either illegally imported from neighboring countries or sourced from antibiotics intended for animal treatment. Numerous challenges arise from uncontrolled use of antibiotics and the most prominent one is occurrence of ARGs in PPB. Particular issue with using antibiotics intended for veterinary purposes on plants is that the active substance is not formulated for plant protection, thus being less efficient when applied. Other problems include the absence of PPP instruction labels, as well asinformation on application frequency and withdrawal period. Consequently, plant growers cannot determine the right concentration of antibiotic, leading to either excessive or insufficient doses during application.

Key words: plant pathogenic bacteria, *Erwiniaamylovora*, streptomycin, antibiotic resistance, horizontal gene transfer

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FORMATION OF SOIL RESISTOME

Simonida Djuric[1*], Timea Hajnal Jafari[1], Dragana Sramenov[1]

[1]University of Novi Sad, Faculty of Agricultura, Novi Sad, Serbia

*simonida@polj.uns.ac.rs

Soil resistome, by definition, represents all the antibiotic resistance genes (ARGs) and related proteins or similar protein likely to evolve toward potent antibiotic resistance in communities of both pathogenic and non-pathogenic bacteria (soilborn or introduced) living in soil as habitat. Such genes, defined as antibiotic resistance when found in pathogens, may serve a very different function for their host in the environment. Of all the environments, soil undoubtedly contains the richest and most diverse populations of microorganisms. The use of antibiotics in all spheres of human activity inevitably leads to their deposition in the soil. The ways of reaching the soil are different: - use of manure, organic fertilizers originating from animal remains, irrigation with water from industries that use antibiotics and so. Once when get in, soil ARGs can spread in various ways, such as horizontal gene transfer (HGT) between soil microorganisms and vertical gene transfer (VGT) between parent and next generation. Accumulation of ARGs in soil due to their high similarity to ARGs in human pathogens has emerged as a major public health problem in recent years. The importance and consequences of the accumulation of ARGs in the soil have stimulated a large number of studies with the aim of understanding the environmental factors that control their abundance and diversity from local to global scales. In recent years, information on soil ARGs, that are part of the soil resistome, are increasingly available in reference databases such as RefSoil+. However, there are still doubts about the exact distribution of ARGs in the soil profile and the way in which all abiotic and biotic factors influence their diversity and representation in the soil. Although many methods were developed to overcome the problem, these methods are insufficient to eradicate antibiotic resistance genes (ARGs) unless their sources, occurrence, and spread mechanisms are fully revealed. However, accurate source-tracking of ARGs and their effects on soil environment has always been confounded. At the end, there is the question - How to prevent the resistome formation in soil?

Key words: resistome, soil, microorgansms AGRs, HGT, VGT

BIOSECURITY ISSUES IN COMMON CARP PRODUCTION

Miloš Pelić[1*], Milica Živkov Baloš[1], Nikola Puvača[2], Nenad Popov[1], Ana Gavrilović[3], Jurica Jug-Dujaković[4], Dragana Ljubojević Pelić[1]

[1]Scientific Veterinary Institute "Novi Sad", Novi Sad, Serbia
[2]Department of Engineering Management in Biotechnology, Faculty of Economics and Engineering Management in Novi Sad, University Business Academy in Novi Sad, Novi Sad, Serbia
[3]University of Zagreb, Faculty of Agriculture, Zagreb, Croatia
[4]Sustainable Aquaculture Systems Inc., Frenchtown, New Jersey, USA

*milosp@niv.ns.ac.rs

Increasing investments in development and research in the field of aquaculture, improvement of genetic potential and increased requirements for fish as a significant source of animal proteins and omega 3 fatty acids, are considered the main reasons for production increase in aquaculture, both on a global and local level. Intensive production practices increase both biosecurity risks and risks for producers. The challenges in the control of diseases in fish are far more complex than the control of diseases in terrestrial animals, primarily due to the aquatic environment, the rapid spread of infection through water, and the impossibility of isolating the sick fish. An alarming problem is the uncontrolled and careless use of antibiotics in ponds, which results in the emergence of antibiotic resistance. The present work aims to provide an overview ofthe main biosecurity issues related to common carp production. Literature research was conducted and data from the available literature were used. There is no doubt that the diseases of cultured carp represent one of the biggest patterns of economic losses in carp production. In addition to the clearly defined challenges faced by carp production, which are extremely important for sustainable and profitable production, carp production also faces challenges and problems that are more difficult to control.Adequately prescribed and implemented preventive measures represent an essential segment of the protection of fish farming systems due to the limited possibilities of treating many diseases, and precisely because of this, a good understanding of the way fish diseases spread is extremely important. It is crucial to identify diseases at an early stage in order to prevent further production losses, both direct and indirect. The challenges and problems of managing good biosecurity are wide-ranging and multifactorial those affect the exposure of farmed stocks and consequently increase the risk of infection with subsequent losses. Anthropogenic challenges are also very common, but often problems can result from the physical location and/or the poor design and construction of new fish farms. Most of the ponds were built during the transition period in the early 1990s.A well-designed fish farm will minimize potential long-term problems. Handling is an indispensable part of all carp culture technologies and, as a very important predisposing factor for diseases. Handling should not be neglected, in order to reduce its negative effects. There is a need for better regulation and health legislation across aquaculture in our country. Due to the lack of precisely prescribed regulatory frameworks and guidelines, it is difficult for fish farmers to apply certain measures, such as maximum population density, to carry out disease surveillance, regular health checks and monitoring, apply biosecurity plans, and finally address to the competent authorities for adequate advice. The focus should be on the development of strategies aimed at preventing infection, with the combined application of good management and good production practices in the first place, then the application of immunoprophylaxis and the implementation of biosecurity measures, and finally controlled use of registered antibiotics. In the end, the application of all these measures can result in effective protection of fish in aquaculture.

Key words: fish diseases, preventive measures, fish pond, common carp, aquaculture

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WASTEWATER FROM SLAUGHTERHOUSES: A RISK FOR ANTIMICROBIAL RESISTANCE DISSEMINATION THROUGHOUT THE ONE HEALTH CONCEPT

Katerina Blagoevska[1*], Gordana Ilievska[1], Biljana Stojanovska Dimzoska[1], Mirko Prodanov[1], Maxud Krluku[2], Marija Ratkova Manovska[1]

[1]University Ss Cyril and Methodius, Faculty of veterinary medicine, Lazar Pop – Trajkov 5-7, 1000 Skopje, Macedonia
[2]Veterinary Practice Maxi, Amdi Leshi 21, 1250 Debar, Republic of North Macedonia

*katerinab@fvm.ukim.edu.mk

Wastewaters present serious danger for the spread of antimicrobial resistance (AMR) across the One Health concept, particularly when considering the daily discharge of billions of liters from households, farms, factories, and institutions. Regardless of their origin, they serve as a reservoir of microorganisms, including both pathogenic and infectious ones, as well as solid waste dominated by toxic, inflammable, and corrosive materials. The likelihood that wastewater, particularly from slaughterhouses contains clinically significant antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) that cannot be completely removed using traditional treatment methods is pretty high. The situation is likely to worsen due to the widespread use of all major classes of antibiotics, such as beta-lactams, macrolides, quinolones, aminoglycosides, tetracyclines, and potentiated sulphonamides, in livestock and poultry farms across various geographic regions. Therefore, in 2017, the United Nations Environmental Assembly (UNEA) acknowledged the environment as a critical factor in the transmission and spread of resistant pathogens and commensal bacteria from various sources. The direct impact on the environment includes contamination and destruction of natural habitats for various plant and animal species, leading to complex health and safety concerns for humans, posing a significant biosafety risk. According to current WHO estimates, about 24% of deaths are attributed to environmental factors. Additionally, the social and economic risks posed by these environmental factors should not be overlooked. Hence, in 2023, in line with the One Health approach, the European Commission proposed that environmental monitoring of AMR in ground waters and surface waters, including coastal waters, wastewater and agricultural soils is essential to further understand the importance of the environment in the emergence and spread of AMR, the levels of environmental contamination and the risks posed to human health. This paper presents data obtained from a biomonitoring of wastewaters from slaughterhouses geographically located in different regions in Macedonia. We targeted presence of *Salmonella, Campylobacter jejuni, Campylobacter coli*, indicator commensal *Escherichia coli (E. coli)*, indicator commensal *Enterococcus faecalis* and *Enterococcus faecium*. The biomonitoring also included *Salmonella spp.* and *E. coli*, producers of: β - lactamase broad spectrum (ESBL); AmpC β-lactamases (AmpC) and carbapenemases. Among the results, the most concerning were the commensal *E. coli* ESBL phenotype, that showed resistance to colistin and *Enterococcus faecalis* (*E. faecalis*) resistant to vancomycin. Among the commensal *E. coli* isolates 40% were Multidrug resistance (MDR), while in *E. faecalis*, 25% were proven to be MDR, showing resistance to chloramphenicol, erytromicin, gentamicin, tertacycline and vancomycin. To reduce the risks associated with slaughterhouse wastewater, several measures are crucial to be undertaken. They comprise: implementing proper wastewater treatment processes, implementing regulations on the antibiotic use in animal breeding, educating farmers, veterinarians, slaughterhouse workers, and the general public, and most importantly, enforcing an obligate monitoring and surveillance program of AMR.

Key words: wastewaters, slaughterhouses, One Health

TOWARD SUSTAINABLE CONTROL OF GRAY MOLD ON STRAWBERRIES: EXPLORING ALTERNATIVES TO SYNTHETIC FUNGICIDES CONSIDERING RESISTANCE EVOLUTION

Marta Loc[1*], Nataša Došen[1,2], Simonida Đurić[1], Tatjana Dudaš[1], Mladen Petreš[1], Dragana Budakov[1], Mila Grahovac[1]

[1]Faculty of Agriculture, University of Novi Sad, Novi Sad, Serbia,
[2]Bacillomix CO d.o.o., Veternik, Serbia

*marta.loc@polj.edu.rs

Graymold, caused by the fungal pathogen *Botrytis cinerea*, is one of the most destructive diseases of small fruit crops and is considered to be a high-risk pathogen in terms of resistance to fungicides. Due to limited options for chemical control, long post-harvest intervals, resistance risks that synthetic fungicide application carries, and the urge forsustainable plant protection, the biocontrol of postharvest diseases such graymold has become a hot spot. In this study, highly potent antimicrobial *Bacillus amyloliquefaciens* strain was tested to evaluate its ability to control graymold in strawberry open field crops. Field trials, set according to EPPO method PP 1/16 (3), were conducted on four localities of Province of Vojvodina–Zrenjanin, Rumenka, Vojvoda Stepa and Pivnice. The effect of the tested agent at concentrations of 0.03%, 0.05% and 0.1% was assessed based on the intensity of mature fruit infection. Assay indicated that the efficacy of the tested agent at application rates of 0.05% and 0.1% ranged from 84.97% to 93.66%, compared to the applied standard agent *Bacillus amyloliquefaciens* strain MBI600, which showed efficacy between 92.25% and 93.06%. This study revealed that the applied biological agent could be used as an effective alternative to synthetic fungicides and suggests the potential for its commercialization as a biofungicide, with low or no risk of resistance development in target pathogen *B. cinerea.*

Key words: graymold, *Botrityscinerea*, biocontrol, sustainability, fungicide-resistance

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FACTORS AFFECTING THE CO-AGGREGATION ABILITY OF PROBIOTIC YEAST *SACCHAROMYCES BOULARDİİ* WITH *CANDİDA* SPP. AND THEIR AUTO-AGGREGATION

Ružica Tomičić[1*], Ivana Čabarkapa[2], Ana Varga[2], Milica Nićetin[1], Biljana Lončar[1], Violeta Knežević[1], Zorica Tomičić[2]

[1]Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia
[2]Institute of Food Technology in Novi Sad, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

*ruzica.tomicic@yahoo.com; ruzica.tomicic@uns.ac.rs

Infections caused by *Candida* species have been increasing dramatically worldwide in recent years, although several strategies for the prevention and treatment of candidiasis have been adopted at the clinical level such as the use of various antifungal drugs. Given this scenario, there are a growing number of potential health benefits attributed to probiotics. Most of the commercialized probiotics contain bacteria, while among yeasts, only *Saccharomyces boulardii* is widely used and represents an effective biotherapeutic agents against *Candida* infections. With this in mind, we investigated the co-aggregation ability of *S. boulardii* with different *Candida* strains as well as their auto-aggregation under different culture conditions such as temperature (28 °C, 37 °C, 42 °C) and pH (4.5, 7.0, 8.5) after 5 h and 24 h. The evaluation of aggregation in this study revealed that all tested yeasts had the ability to aggregate, which increased significantly over a period of 24 h for all combinations of probiotic and *Canida* strains. It was evident that *C. albicans* and *C. krusei* followed by the probiotic strain *S. boulardii* showed the highest degree of auto-aggregation, whereas the lowest was noted in *C. glabrata* strains. On the other hand, co-aggregation between probiotic *S. boulardii* and *Candida* strains was strain-specific. The presence of the probiotic strain *S. boulardii* significantly inhibited the aggregation of *C. albicans* ATCC 10261, *C. krusei* ATCC 6258, and *C. glabrata* ZIM 2369, while in the case *C. glabrata* ZIM 2382 aggregation was even enhanced. Regarding the influence of different culture conditions, such as temperature and pH, the results showed that these factors also affect the aggregation ability in a different way only after 5 h of incubation, with the highest cell aggregation recorded at a temperature of 37 °C in most cases and pH 4.5. Taking into account the fact that probiotic *S. boulardii* has the ability to reduce the aggregation of *Candida* spp., our experimental findings provide new insight for the use of *S. boulardii* in the battle against *Candida* infections.

Key words: aggregation, *Saccharomyces boulardii*, *Candida* spp., temperature, pH

ANTIBACTERIAL ACTIVITY OF ESSENTIAL OILS AND MAIN COMPOUNDS AGAINST *SALMONELLA* TYPHIMURIUM AND *STAPHYLOCOCCUS AUREUS*

Zorica Tomičić[1*], Ljubiša Šarić[1], Ivana Čabarkapa[1], Ana Varga[1], Dragana Plavšić[1], Olja Todorić[1], Ružica Tomičić[2]

[1]Institute of Food Technology in Novi Sad, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

[2]Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

*zorica.tomicic@fins.uns.ac.rs

The growing challenge of microbial resistance to many antimicrobial agents has prompted the search for alternative sources of antimicrobial agents, which are believed to be found abundantly in plants. Plant essential oils, due to the large biological and structural diversity of their compounds, are known to have many potential benefits. The aim of this study was to evaluate the antibacterial and anti-adhesion activity of fifteen essential oils and their compounds against two bacterial species *Salmonella Typhimurium* ATCC 25923 and *Staphylococcus aureus* ATCC 14208 responsible for food spoilage and infectious disease. Antibacterial activity was determined by testing the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of essential oils and compounds. The method used to assess adhesion to polystirene was crystal violet staining. The results showed that MIC and MBC values ranged from 0.31 to 320 mg/mL and 0.63 to 320 mg/mL for the tested strains, respectively. Planktonic cells of bacteria showed widevariability in resistance to essential oils, with *Cinnamomum zeylanicum* and *Eugenia caryophyllus* being the most effecttive, followed by *Origanum vulgare*, *Thymus vulgaris* and*Ocimum basilicum*. The activity was more pronounced against gram-positive bacteria than gram-negative bacteria. Based on the percentage of adhesion inhibition, *E. caryophyllus* essential oil showed the strongest effect with a percentage of inhibition of 53.5 and 73% on *S*. Typhimurium ATCC 14208 and *S. aureus* ATCC 25923, respectively. On the other hand, the anti-adhesive properties of the essential oils of *Eucalypti aetheroleum* and *Salvia officinalis* were significantly lower than the others. According to the results of antimicrobial susceptibility, the most active compounds were carvacrol and thymol, which showed the most remarkable anti-adhesion effect against these bacterial strains with a percentage of inhibition up to 66%. This research should help to clarify the application of these essential oils for the treatment of many infectious diseases and food preservation in the future.

Key words: essential oils, antibacterial agents, anti-adhesion agents, *Salmonella* Typhimurium, *Staphylococcus aureus*

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EVALUATION OF TIAMULIN'S ANTIBACTERIAL ACTIVITY ON CLOACAL MICROFLORA, AND PHARMACOKINETICS FOLLOWING SINGLE AND MULTIPLE ORAL ADMINISTRATIONS IN GEESE

Charbel Fadel[1,2,3], Mario Giorgi[1,3], Irene Sartini[1,3], Beata Lebkowska-Wieruszewska[4], Filomena Dessì[1], Firas Serih[1], Amnart Poapolathep[5], Cristina Vercelli[6]*

[1]Department of Veterinary Medicine, University of Sassari, Sassari, Italy.
[2]Department of Veterinary Medicine, Lebanese University, Beirut, Lebanon
[3]Department of Veterinary Sciences, University of Pisa, San Piero a Grado, Pisa, Italy.
[4]Department of Pharmacology, University of Life Sciences, Lublin, Poland.
[5]Faculty of Veterinary Medicine, Department of Pharmacology, Kasetsart University, Thailand.
[6]Department of Veterinary Sciences, University of Turin, Torino, Italy

*cristina.vercelli@unito.it

Tiamulin is a semi-synthetic antibiotic approved exclusively in veterinary medicine, active against Gram-positive bacteria as well as *Mycoplasma* spp. and *Leptospirae* spp. The study aims to evaluate drug effects on resistance in cloacal flora in an in vivo experimental trial in geese andestablish tiamulin's pharmacokinetic features. Eight healthy geese underwent a longitudinal study divided into two phases (60 mg/kg single oral administration vs 60 mg/kg/day for four days), with a wash-out period of two weeks. Blood samples and cloacal swabs were collected at pre-assigned times. Tiamulin levels in goose plasma were quantified using a fully validated HPLC method coupled with a UV detector. Cloacal swabs were employed to identify bacterial strains, employing species-specific methods and affirmative procedures. The Minimal Inhibitory Concentration (MIC) was determined for each isolated bacterial species. Tiamulin exhibited a slow to moderate terminal half-life (3.13 hr single; 2.62 hr multiple) and rapid absorption (1 hr single; 0.5 hr multiple) in geese following oral administration, with an accumulation ratio of 1.8 after multiple doses. The cloacal isolation allowed the identification of various bacterial strains, all commensal. In both treatments, The MIC values were very high, demonstrating a resistance against tiamulin that can be present before the initial administration for some strains or emerge shortly after the commencement of treatment for others. While these isolates form part of the commensal flora, the potential for cross-resistance, environmental spread, and transformation into opportunistic pathogens should not be overlooked. Addressing these challenges may involve further research exploring tiamulin efficacy against *Mycoplasma* species and other key pathogens *in vivo*, in the diseased state, optimizing tiamulin's dosing regimen accordingly, and potentially considering synergistic and rotation treatments. This strategic approach is especially crucial in addressing antibiotic resistance for commensal bacteria outside TMC's spectrum of action, thereby preserving balance within the microbial community while ensuring effective targeting of intended pathogens.

Key words: antibacterial, commensal flora, goose, pharmacodynamics, pharmacokinetics, tiamulin

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COMPARATIVE EFFICACY OF INTRAMAMMARY PEG-PROPOLIS FORMULATION VERSUS AMOXICILLIN FOR MASTITIS TREATMENT IN GOATS

Jelena Šuran[1*], Lada Radin[2], Jasna Aladrović[2], Blanka Beer Ljubić[3], Nino Mačešić[4], Goran Bačić[4], Tomislav Mašek[5], Josipa Vlainić[6], Antun Kostelić[7]

[1]ApiotixTechnologies, Split, Croatia
[2]Department of Physiology and Radiobiology, Faculty of Veterinary Medicine, University of Zagreb, Croatia
[3]Internal Diseases Clinic, Faculty of Veterinary Medicine, University of Zagreb, Croatia
[4]Reproduction and Obstetrics Clinic, Faculty of Veterinary Medicine, University of Zagreb, Croatia
[5]Department of Animal Nutrition and Dietetics, Faculty of Veterinary Medicine, University of Zagreb, Croatia
[6]Division of Molecular Medicine, Institute RuđerBošković, Zagreb, Croatia
[7]Department of Animal Science, Faculty of Agriculture, University of Zagreb, Croatia

*jelena@apiotix.eu

This study aimed to evaluate the efficacy of a polyethylene glycol (PEG)-propolis formulation for intramammary administration as an alternative to conventional antibiotic therapy in managing mastitis in goats. The objectives included performing chromatographic profiling of our formulation to identify ten markers of biological activity; assessing the antimicrobial efficacy of this formulation against prevalent mastitis pathogens *in vitro*; comparing the efficacy of the PEG-propolis formulation with that of amoxicillin, including clavulanic acid, for bacteriological clearance; and examining the effects of both treatments on oxidative stress markers in milk from affected mammary glands. Ten markers of biological activity were identified and quantified with HPLC-UV. The PEG-propolis formulation exhibited significant antimicrobial efficacy *in vitro*, especially against *S. aureus*, achieving microbial inhibition and biofilm eradication at concentrations lower than the experimental 3%. The efficacy studyincluded 25 goats diagnosed with subclinical mastitis from two alpine goat dairy farms, leading to a distribution of 15 infected udders in the antibiotic group and 20 infected udders in the propolis group. Pathogen identification in the goat-infected udders revealed the presence of *Staphylococcus* spp., *Staphylococcus aureus*, *Streptococcus* spp., *S. uberis*, and *Arcanobacterium pyogenes*. The goats were divided into two groups for treatment: one group received a 3% PEG-propolis formulation, while the other was treated with intramammary amoxicillin and clavulanic acid, administered three times, 12 hours apart. The treatment led to a 75% cure rate within the first 7 days, increasing to 85% by the 14th day, surpassing the antibiotic treatment's 73.3% cure rate. Moreover, the propolis treatment significantly enhanced the antioxidative response in the milk, with the highest activity of glutathione peroxidase (GSH-Px) recorded at 581 ± 193 U/L 12 hours after treatment, compared to the highest level of GSH-Px following antibiotic treatment at 344 ± 167 U/L, observed 24 hours post-treatment. These findings support the PEG-propolis formulation as a potent and biologically favorable alternative to antibiotics for mastitis treatment in goats, offering efficacious bacteriological healing and significant antioxidative benefits without the need for conventional antibiotic therapy.

Key words: mastitis, goats, propolis, amoxicillin

IMPLICATION FOR HUMAN CLINICAL SETTINGS OF A RESTRICTIVE INTERVENTION ON 3RD GENERATION CEPHALOSPORINE (3GC) USE IN DAIRY FARMS

Beatriz Llamazares[1*], Matthew Avison[1], Winnie W. Y. Lee[1], Phillip Williams[2]

[1]Cellular and Molecular School, University of Bristol, Bristol, UK
[2]Bristol Royal Infirmary, Bristol, UK

*beatriz.llamazares@bristol.ac.uk

3GCs are important therapeutics for humans and also prescribed for animals. In June 2018, restrictions were introduced on UK dairy farms almost eliminating their use. We hypothesised that this would cause a reduction in CTX-M-mediated but not AmpC-mediated 3GC resistance (3GC-R), because the latter also confers resistance to amoxicillin-clavulanate, where usage was not restricted. 828 faecal samples were collected on 20 South-West English dairy farms in 2017/18 (Pre intervention) and 645 samples in 2020/21 (Post-intervention). 3GC-R *E. coli* where isolated by selective plating. 3GC-R *E. coli* was isolated from human bloodstream infections in the same geographical area in 2017-18 (n=68) and 2020 (n=67). Whole genome sequencing (WGS) was used to identify 3GC-R mechanisms. Pre-intervention, the ratio of CTX-M to AmpC mediated 3GC-R in farm *E. coli* was 50:50; post-intervention it had shifted to 37:74 in favour of AmpC, as predicted. In contrast, the ratio was 90:10 in favour of CTX-M in human bloodstream isolates. Notably, while blaOXA-1 (an alternative cause of amoxicillin-clavulanate resistance) was never associated with blaCTX-M in 3GC-R farm isolates, 56% of blaCTX-M positive human isolates also carried blaOXA-1. CTX-M-positive 3GC-R farm isolates are rarely amoxicillin-clavulanate resistant therefore reducing 3GC use whilst maintaining amoxicillin-clavulanate use has shifted 3GC-R towards an AmpC-mediated mechanism, which is mutational, and non-transmissible. However, this might not translate to human settings because a majority of CTX-M-positive isolates are 3GC/amoxicillin-clavulanate dual resistant due to CTX-M/OXA-1 linkage. Hence reductions in 3GC and amoxicillin-clavulanate may be necessary to reduce the burden of CTX-M-positive *E. coli* in the human population.

Key words: 3GC usage reduction, amoxicillin-clavulanate, 3GC resistance, CTX-M mechanisms, AmpC hyper-production, bla-OXA, *Escherichia coli*, whole genome sequencing, dairy farms, human blood stream infections

DOES RESISTANCE TO 3RD /4TH GENERATION CEPHALOSPORINS AND FLUROQUINOLONES ALONG WITH THEIR MOLECULAR MECHANISMS CHANGE WHEN WE STOP USING THEM? A LONGITUDINAL STUDY ON *ESCHERICHIA COLI* FROM 20 UK FARMS

Beatriz Llamazares Bayon[1], Matthew Avison[1], Kristen Reyher[2], Elliot Stanton[2]

[1]Cellular and molecular School, University of Bristol, Bristol, UK
[2]Veterinary School, University of Bristol, Bristol, UK

*beatriz.llamazares@bristol.ac.uk

Highest-priority critically important human antibacterials (HP-CIAs) 3rd/4th generation cephalosporins (3GC/4GC) and fluoroquinolones are also used in farmed animals to treat bacterial infections. In June 2018, regulations through farm assurance were introduced on UK dairy farms almost eliminating the use of these medicines. This presented an excellent opportunity to study the effect of this development on the levels and molecular ecology of HP CIA resistance using longitudinal surveillance. *Escherichia coli* is found in the large intestine of both animals and humans. This bacterium can cause serious opportunistic infections in humans and can be a reservoir for transferable antibacterial-resistant genes. Most relevant to this project was the dominant extended-spectrum β-lactamase type, CTX-M, which confers resistance to 3GC/4GC (3/4GC-R). We have previously demonstrated that 4GC use was associated with CTX-M positivity among *E. coli* from calves on UK dairy farms. Furthermore, fluoroquinolone use was associated with fluoroquinolone resistance (FQ-R) and amoxicillin/clavulanate use was associated with AmpC-mediated 3GC-R, since AmpC β-lactamases also confer amoxicillin/clavulanate resistance. 825 samples (calves n=270, cows n=555) were collected from faecally contaminated sites on 20 dairy farms in South West England between April 2017 and December 2018 (pre intervention); 651 samples (calves n=551, cows n=100) were collected from the same farms between August 2020 and October 2021 (post-intervention). 3/4GC-R and FQ-R isolates were identified by selective plating. PCR and short read Illumina WGS were used to identify 3/4GC R mechanisms. Mixed effect logistic regression modelling with farm as a random effect was used to assess changes. FQ-R *E. coli* positivity did not change significantly in calves (pre-regulations=17%, post-regulations=20% $p=0.36$) or cows (pre-regulations=4.5%, post-regulations=7% $p=0.06$) despite almost zero sales of FQs to these farms since June 2018. In contrast, 3/4GC-R positivity reduced post-regulations in calves (pre-regulations=27%, post-regulations=19%, $p<0.00001$ but not in cows pre-regulations=7%, post-regulations=8%, $p=0.42$). This reduction in positivity in calves coincided specifically with a reduction in the prevalence of CTX-M-positive *E. coli* (pre-regulations=12.5%, post-regulations=7% $p<0.00001$) although a similar reduction in CTX-M-positive *E. coli* was not seen in cows (pre-regulations=5%, post-regulations=0%, $p=0.16$.CTX-M prevalence was associated with a reduction of 3/4GC usage on dairy farms, but other interventions (or more time) may be required to reduce AmpC-mediated 3GC-R and FQ-R.

Key words: 3rd/4th generation cephalosporins resistance, fluoroquinolone resistance, restriction usage, CTX-M mechanisms, AmpC-hyperproduction mechanisms, *Escherichia coli*, whole genome sequencing, dairy farms

A NEW CONCERN: MABR – MYCOTOXIN-INDUCED ANTIBIOTIC RESISTANCE

Ksenija Nešić[1*], Nemanja Zdravković[1], Vladimir Radosavljević[1]

[1]Scientific Institute of Veterinary Medicine of Serbia, Belgrade, Serbia

[*]ksenija.nesic@nivs.rs

The presence of mycotoxins in food and feed represents one of the major safety concerns today, comparable in significance and worldwide prevalence to antimicrobial resistance. Recently, there has been increased attention to the interconnectedness of these two issues, with growing acknowledgment and reduced skepticism regarding such connections. Firstly, both mycotoxins and certain antibiotics are produced by fungi, blurring the distinction between them, as many mycotoxins possess antibacterial properties. Beyond their shared origin, they also exhibit similarities in other characteristics (biosynthesis, structures, genetic composition, and roles within the producing organism's life cycle). Moreover, induction of similar resistance mechanisms in bacteria, supports the notion that antimicrobial resistance caused by mycotoxins is plausible. The inevitable presence of mycotoxins in animal feed, often in trace amounts, mimics the exposure of commensal bacteria in domestic animals to low-level antibiotic growth promoters. Chronic sub-dosing of antibiotics in animals is a recognized contributor to the emergence of antibiotic-resistant bacteria in humans. Another connection between mycotoxins and antibiotics regarding antimicrobial resistance is that bacteria resistant to mycotoxins could transfer resistant genes to other bacteria through horizontal gene transfer. Equally significant is the detrimental impact of both mycotoxins and antibiotics on animal gut microbiota, leading to dysbiosis. Clinically, misdiagnosing mycotoxicosis—diseases caused by mycotoxins that are not treatable with antibiotics—can result in inappropriate and unwarranted antibiotic therapy, exacerbating antimicrobial resistance. Failure to recognize mycotoxins as the primary cause of clinical or production issues in livestock often leads to misguided treatment approaches. Considering these points, it is imperative to conduct further research on mycotoxin-induced antibiotic resistance (MABR), particularly regarding the types of mycotoxins, bacterial species involved, potential combinations of exposures, etc. This issue should not be overlooked and warrants greater attention and investigation.

Key words: antimicrobials, bacterial resistance, fungal products

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THE ROLE AND IMPORTANCE OF BOVINE LACTOFERRIN AGAINST ANTIBIOTIC-RESISTANT MASTITIS PATHOGENS

Annamaria Galfi Vukomanović[1*], Miodrag Radinović[1], Ivana Davidov[1], Zorana Kovačević[1], Sandra Nikolić[1], Mihajlo Erdeljan[1], Tijana Kukurić[1], Jovan Stanojević[1]

[1]University of Novi Sad, Faculty of Agriculture, Department of Veterinary Medicine, Novi Sad, Serbia

[*]annamariagalfi@gmail.com

The World Health Organization has launched its "Global action plan on antimicrobial resistance" due to serious concerns about drug resistance in microorganisms. The aim of this plan is to reduce the overuse of antibiotics and to find novel antimicrobial drugs. Mastitis is a complex and common disease of dairy cows causes severe economic losses and mainly caused by contagious and environmental bacterial pathogens. Treatment of mastitis includes a preventive and therapeutic measure which primarily relies on the antibiotics use. In veterinary medicine, most antibiotics are used inappropriate or unnecessary to prevent mastitis which led to increase the risk for development of antimicrobial resistance. Since treatment of mastitis using antibiotics is problematic, lactoferrin as a natural protein may offer an alternative in mastitis treatment. The objective of this paper is to indicate the importance of bovine lactoferrin in host defense and its possible application against antibiotic-resistant bacteria. Lactoferrin is the main whey glycoprotein present in milk from different dairy animals. Concentration of lactoferrin is quite variable and depends on the stage of lactation and mammary gland health status. Bovine lactoferrin has many physiological and biological functions, including antimicrobial and anti-inflammatory activities. Lactoferrin can reduce bacterial growth and inhibit bacterial adhesion and biofilm formation, thus, it should be considered as a helpful antimicrobial therapeutic agent. Lactoferrin shows antimicrobial activity against many bacteria: *Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli*, coagulase-negative staphylococci, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. The antibacterial activity of lactoferrin against mastitis pathogens depends primarily on its concentration in milk and the degree of iron saturation of the molecule. Lactoferrin is able to enhance the antibacterial effect of antibiotics by damaging the bacterial membrane.

Key words: bovine lactoferrin, antibiotic-resistant pathogens, mastitis, cow

BACTERIAL INFECTIONS IN FRESHWATER AQUACULTURE WITH REFERENCE TO THE CURRENT PROBLEM OF ANTIMICROBIAL RESISTANCE

Dušan Lazić[1*], Miloš Pelić[1], Marko Pajić[1], Dalibor Todorović[1], Zoran Ružić[2], Nikolina Novakov[2]

[1]Scientific Veterinary Institute Novi Sad, Serbia
[2]Department of Veterinary Medicine, Faculty of Agriculture, Novi Sad, Serbia

*dusan.l@niv.ns.ac.rs

Infections caused by motile aeromonads are ubiquitous and are associated with several species and subspecies including *Aeromonas hydrophila, Aeromonas caviae, and Aeromonas sobria*. These bacteria are opportunistic pathogens and cause diseases in freshwater (less commonly marine) fish worldwide. Despite their undisputed high prevalence, the fact that infection with motile aeromonads is often a secondary infection often diminishes its significance. However, the presence of this infection in fish farms, especially if the causative agent is *A. hydrophila*, should not be ignored, as although it may not be a primary pathogen, this infection is the most common factor significantly affecting fish morbidity and mortality. In addition to the mentioned infections with *A. hydrophila*, other bacterial agents of fish diseases such as bacteria of the genus *Flavobacterium, Pseudomonas*, and mycobacteriosis are also present. Also, the fact that members of the motile aeromonad group occur worldwide and affect a large number of fish species in different freshwater environments makes this disease an important factor in aquaculture and open waters. Due to the occurrence of numerous viral diseases in domestic aquaculture in fish farms and other fish farming systems, the incidence of secondary bacterial infections in fish has drastically increased as a result. Consequently, the uncontrolled and unprofessional use of antimicrobial drugs for the prevention and treatment of fish from these bacterial and viral infections has increased. All of this has collectively resulted in a certain degree of resistance of bacterial pathogens of fish diseases to certain antimicrobial therapeutic agents. In aquaculture, as well as in other animal farming areas, antibiotics are used for the prevention and treatment of infections. Some of the common types of antibiotics used in aquaculture, expressed as percentages in relation to their use and prevalence, are: Tetracyclines (30-40%), Florfenicol (20-30%), Oxytetracycline (20%), Sulfonamides (10-15%), Fluoroquinolones (5-10%), Nitrofurans (up to 5%). The prevalence of resistance in aquaculture varies depending on the classes of antibiotics used and is often proportional to the prevalence of antimicrobial drug use in the production process.

Key words: Infections, freshwater aquaculture, motile aeromonads, pathogens, secondary bacterial infections, control, prevention, antimicrobial therapeutic agents

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CHEMICAL COMPOSITION OF CARDAMOM (ELETTARIA CARDAMOMUM (L.) MATON, ZINGIBERACEAE) ESSENTIAL OIL

Maja Hitl[1,2*], Katarina Bijelić[1,2], Katarina Radovanović[1], Blagoje Prpa[1,2], Nebojša Kladar[1,2]

[1]Department of Pharmacy, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia
[2]Center for Medical and Pharmaceutical Investigations and Quality Control (CEMPhIC), Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia

*maja.bekut@mf.uns.ac.rs

Cardamom (*Elettaria cardamomum* (L.) Maton, Zingiberaceae) represents a plant native for Indian subcontinent. The pods are used both for culinary and traditional medicinal purposes. This herbal drug is often characterized as "queen of spices" and represents the third most expensive spice in the world. Main active principle present of this herbal drug is the essential oil (EO), and its content can be characterized as high, as it can go up to nearly 15%. Cardamom is used in traditional medicine, as it shows positive effects on the digestive and respiratory tract, it can be used as aid in regulating blood sugar levels, but it also demonstrates antimicrobial activity in various infections. The aim of the study was to investigate the chemical composition of cardamom's EO. The EO was isolated from previously grounded herbal drug by hydrodistillation. The chemical characterization of compounds was performed using gas chromatography (GC) coupled to a mass spectrometry detector (MS). The yield of extraction was 2.06% (m/m). The most abundant group of compounds were esters of monoterpens (61.83% of total content), followed by oxygenated monoterpenes (25.67%) and monoterpene hydrocarbons (9.10%). Regarding the specific compounds, the EO's most abundant compound was monoterpenic ester α-terpinyl acetate (57.50% of total content), followed by oxygenated monoterpenes eucalyptol (14.63%) and α-terpineol (6.50%), suggesting the potential use in the treatment of infections, including infectious diseases of the skin, respiratory tract and urinary system.

Key words: cardamom, essential oil, GC-MS, terpinyl acetate

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CANNABINOID CONTENT IN HEMP-BASED COSMETIC PRODUCTS

Katarina Bijelić[1,2*], Blagoje Prpa[1,2], Katarina Radovanović[1], Branislava Srđenović Čonić[1,2], Maja Hitl[1,2], Nebojša Kladar[1,2]

[1]University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Hajduk Veljkova 3, 21000 Novi Sad, Republic of Serbia
[1]University of Novi Sad, Faculty of Medicine, Center for Medical and Pharmaceutical Investigations and Quality Control, Hajduk Veljkova 3, 21000 Novi Sad, Republic of Serbia

*katarina.bijelic@mf.uns.ac.rs

Products obtained from hemp, such as oil and various extracts, are often the starting material for the production of dermocosmetic preparations. As the number of skin diseases is constantly increasing, it has been shown that hemp oil has the potential to be used in treatment of pruritus, skin cancer, and inflammatory skin diseases. Cannabidiol (CBD), which is highly abundant cannabinoid in hemp oil, was shown to act as an antimicrobial agent, and could reduce the use of commercially available antibiotic topical preparations, thus lowering the contribution to the development of antimicrobial resistance.As the obtained CBD oils often use outdated extraction methods, that contain non-negligible concentrations of Δ^9-THC, which questions their health safety, and exposes consumers to possible health risks resulting from dermal exposure. The aim of this research was to determine the content of Δ^9-THC, CBD and cannabinol (CBN) in cosmetic products based on hemp oiland to examine the health safety of the same product in terms of the content of "total" Δ^9-THC. Samples of six cosmetic products based on hemp (1g) were extracted with 2 mL of internal standard (ketamine hydrochloride) in acetonitrile (concentration 0.2 μg/mL). The extraction was performed in an ultrasonic bath for 15 minutes. After extraction, mixturewas centrifuged, the supernatant was transferred to a vial for gas chromatography and analyzed using a previously developed and validated GC-MS method. The content of CBD in the examined samples was in the range 1.43-12.82 μg/g of the product. The THC content in two samples was lower than the detection limit, while in the others it was in the range of 4.29-7.25 μg/g of product. CBN was detected in only one of the samples with values of 1.47 μg/g of the product. According to Federal Institute for Consumer Health Protection and Veterinary Medicine (BgVV), only hemp oil that complies with the guidelines for edible oils obtained from industrial hemp seeds (5 mg/kg) should be used in cosmetic products, which indicates that one of the tested products does not meet the requirements of this guideline in terms of THC content. The results of the conducted research showed that the amount of cannabinoids in cosmetic products varies significantly. In certain cosmetic products, the content of "total" Δ^9-THC may represent a health risk, however, it would be necessary to additionally examine the bioavailability of THC from these products, in order to be able to claim with certainty that these products are unsafe. The benefits of these products due to the presence of CBD and emollient components should also be considered before these products are dismissed as unsafe due to the presence of THC.

Key words: hemp, cosmetics products, Δ^9-THC

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CHEMICAL ANALYSIS AND BIOLOGICAL POTENTIAL OF COTTON LAVENDER EXTRACT (*SANTOLINA CHAMAECYPARISSUS* L., ASTERACEAE)

Katarina Radovanović[1*], Nebojša Kladar[1], Milica Aćimović[2], Maja Hitl[1], Katarina Bijelić[1], Blagoje Prpa[1], Neda Gavarić[1]

[1]University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Hajduk Veljkova 3, Novi Sad, Serbia
[2]Institute of Field and Vegetable Crops Novi Sad, National Institute of the Republic of Serbia, MaksimaGorkog 30, Novi Sad, Serbia

*katarina.radovanovic@mf.uns.ac.rs

Santolina chamaecyparissus is a Mediterranean aromatic plant with a great potential for cultivation. It is traditionally used as part of tea mixtures in diabetes treatment. Also, this powerful plant possesses high antimicrobial activity. It was found that the essential oil of *S. chamaeciparissus* has a strong antifungal effect against *Candida albicans* species. In addition, the essential oil of this species strongly inhibits the growth of *Klebsiella pneumoniae* and moderately inhibits several other strains of bacteria (*Bordetella bronciseptica*, *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Staphilococcus aureus* and *Staphilococcus epidermidis*). The aim of this study was toexaminate chemical composition, phenolic and flavonoid content, antioxidant and antihyperglycemic potential. In the cotton lavender ethanolic extract thetotal phenolic content was determined by the Folin-Ciocalte method. The total flavonoid content was determined spectrophotometrically, where the formation of complexes with aluminium ions was monitored. Chemical characterization was performed by HPLC (*High performance liquid chromatography*) analysis. Antioxidant activity wasdetermined by the neutralization of 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl (OH), nitroso (NO) radicals, as well as the process of inhibiting lipid peroxidation. Antihyperglycemic activity was determined *in vitro* by inhibition of α-amylase and α-glucosidase enzymes. The extraction yield was about 7%. Total phenolic content was 96.04 mg GAE (*gallic acid equivalent*)/g d.e. (dry extract). Total flavonoid content was 15 mg QE (*quercetin equivalent*)/g d. e. The presence of multiple compounds was determined by HPLC analysis. Ferulic and *p*-coumaric acid were the most abundant. In *in vitro* antioxidant tests, the half maximal inhibitory concentration was 17.38 μg/ml for DPPH, 203.14 μg/ml for NO and 203.06 μg/ml for OH radical. For the lipid peroxidation inhibition process, that value was 716.03 μg/ml. Inhibition of 50% of α-amylase enzyme activity was achieved at a concentration of 555.2 μg/ml for α-amylaseand 61.55 μg/ml for α-glucosidase. *Santolina chamaecyparissus* is a good source of phenolic compounds. *In vitro* tests showed that the plant possess antihyperglycemic and antioxidant potential. The potential mechanism of the antihyperglycemic effect is the inhibition of the enzyme α –glucosidase. Cotton lavender certainly possess antimicrobial activity, so its multiple activity could be a very significant in different treatment aspects. Due to the results of this study and the previously determined antimicrobial activity, it can be concluded thatits multiple activity could be a very significant in different treatment aspects.

Key words: *Santolina chamaecyparissus*, phenols, ferulic acid, antihyperglycemic effect

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ADDRESSING FUNGICIDE RESISTANCE CHALLENGES: A NOVEL BIOAGENT FOR SUSTAINABLE CONTROL OF ALTERNARIA APPLE FRUIT ROT

Tatjana Dudaš[1*], Aleksandra Delić[1], Mladen Petreš[1], Marta Loc[1], Mila Grahovac[1], Vera Stojšin[1], Dragana Budakov[1]

[1]Faculty of Agriculture, University of Novi Sad, Novi Sad, Serbia

*tatjana.dudas@polj.edu.rs

Alternaria fruit rot is one of the major diseases of apple fruits, causing significant yield losses and reducing quality of apple fruit due to mycotoxin production. The management of this disease relies on the use of fungicides, but alternative measures, such as biocontrol agents, are being promoted as sustainable solutions, overcoming the environmental and health risks of fungicide usage, as well as resistance development in pathogens. In this study, the ability of *Pichia caribbica* yeast strain to control Alternaria fruit rot was tested in orchard trials. The trials were conducte daccording to EPPO method *PP 1/18 (3)* on two localities in Vojvodina Province–Titel and Mala Remeta. The suspension of biocontrol agent (10^7 cells/ml) was applied in two different treatments: two and seven days before harvest, while standard fungicide (fludioxonil) was applied fourteen days before harvest. After five months of cold storage and 14 days at room temperature, apple fruits were scored for disease incidence. Efficacy of the bioagent ranged from 80% at Titel, where it was at the same level of statistical significance as the standard (100%), to 93.75% at Mala Remeta, where it was statistically higher than the standard (62.5%). The results indicate the potential of this bioagent to effectively control the Alternaria fruit rot in the field conditions. This bioagent could serve as a sustainable alternative to fungicides, posing no threat to the environment and human health while addressing the issue of fungicide resistance development in populations of the pathogen.

Key words: Alternaria fruit rot, biocontrol, sustainability, fungicide-resistance

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ESSENTIAL OILS IN COMBATING ANTIMICROBIAL RESISTANCE OF FOODBORNE PATHOGENIC BACTERIA

Suzana Vidaković Knežević[1*], Slobodan Knežević[1], Jelena Vranešević[1], Zoran Ružić[2], Dalibor Todorović[1], Maja Velhner[1], Dubravka Milanov[1], Sunčica Kocić-Tanackov[3], Nedjeljko Karabasil[4]

[1]Scientific Veterinary Medicine "Novi Sad", Novi Sad, Republic of Serbia
[2]University of Novi Sad, Faculty of Agriculture, Department of Veterinary Medicine, Novi Sad, Republic of Serbia
[3]University of Novi Sad, Faculty of Technology Novi Sad, Novi Sad, Republic of Serbia
[4]University of Belgrade, Faculty of Veterinary Medicine, Belgrade, Republic of Serbia

*suzana@niv.ns.ac.rs

Population growth affects the global food supply. With the demand for higher yields in the production of animal products for human consumption, an excessive amount of antibiotics is used. The use of antibiotics is not only as pharmaceuticals, but also as prophylactic agents and antibiotic growth promoters. Irrational use of antibiotics in food production and human health is one of the major factors leading to emergence and spread of antimicrobial resistance. Alternatives to minimize the use of antibiotics and combat antimicrobial resistance are essential oils. Essential oils are natural, liquid, volatile, and complex mixtures of biologically active substances that are formed by aromatic plants as secondary metabolites by all plant organs (roots, stems, bark, leaves, flowers, seeds). In the food production, essential oils like garden angelica (*Angelica archangelica*), basil (*Ocimum basilicum* L.), cinnamon (*Cinnamomum zeylanicum* Nees.), lavender (*Lavandula angustifolia*), lemon (*Citrus limonum*), black pepper (*Piper nigrum*), rosemary (*Rosmarinus officinalis* L.), oregano (*Origanum vulgare*), sage (*Salvia officinalis* L.), and thyme (*Thymus vulgaris*) are commonly used as flavoring agents. In our study, the aforementioned essential oils have shown antibacterial effects against some of the most common foodborne pathogenic bacteria, like *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Listeria monocytogenes*, and *Yersinia enterocolitica*. Concentrations of essential oils needed to combat foodborne pathogenic bacteria were determined by disk diffusion and broth microdilution methods. Among the above-mentioned essential oils, the most active essential oils were oregano (MIC $\leq$ 0.71 μL/mL), thyme (MIC $\leq$ 0.89 μL/mL), and cinnamon (MIC $\leq$ 0.89 μL/mL). To observed the morphological changes of *Salmonella Enteritidis, Salmonella Typhimurium*, *Listeria monocytogenes*, and *Yersinia enterocolitica* cells treated with oregano and thyme essential oils the scanning electron microscopy were used in our study. The treated bacterial cells showed damage, with deformed shapes, rough surfaces, and membrane rupture. The results from this study confirm that selected essential oils are efficient at low concentrations against both Gram-negative and Gram-positive foodborne bacteria. The complex chemical composition of essential oils makes them valuable agents in the treatment of antimicrobial resistance. Essential oils combat foodborne pathogenic bacteria through few different mechanisms of action. The lipophilic essential oils penetrate through membrane into the bacterial cell, causing disruption of cell integrity and increasing the permeability of cell membranes. This leads to decrease in the gradient across the membrane, proton collapse, inhibition of electron transfer, oxidation and loss of proteins, lipids, ribosomes, energy-carrying molecule adenosine triphosphate, and other cellular components. Several previous studies have demonstrated that essential oils can interfere with the expression of genes encoding efflux pumps – one of the main mechanisms of AMR. Large loss of cellular components consequently leads the bacterial cell to death. In conclusion, there are plenty of possibilities for the essential oils to be used in food production, and to serve to combat the most common foodborne pathogenic bacteria.

Key words: *Salmonella* spp., *Listeria monocytogenes*, *Yersinia enterocolitica*, essential oils, antimicrobial resistance

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QUALITY CONTROL OF COMMERCIALLY AVAILABLE MINTH ESSENTIAL OILS

Blagoje Prpa[1,2*], Katarina Bijelić[1,2], Maja Hil[1,2], Katarina Jeremić[1], Nebojša Kladar[1,2]

[1]University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Hajduk Veljkova 3, 21000 Novi Sad, Republic of Serbia

[2]University of Novi Sad, Faculty of Medicine, Center for Medical and Pharmaceutical Investigations and Quality Control, Hajduk Veljkova 3, 21000 Novi Sad, Republic of Serbia

*blagoje.prpa@mf.uns.ac.rs

The *Mentha* species how a long history of usage in traditional medicine. Peppermint essential oil has been shown to be effective against a large number of pathogens. Menthol, the main chemical compound isolated from mint essential oil, is responsible for the antimicrobial effect of essential oils of the genus *Mentha*. Since we live in an era of increasing bacterial resistance, it is necessary to constantly pursue for alternative antimicrobial agents. Due to its antimicrobial activity, peppermint essential oil can be a potential agent that can mittigate this issue since mentholcan act as a bactericidal agent. However, the presence of toxic compounds, like pulegone and menthofuran, highlights the importance of strict quality control. The market's exponential growth raises concerns about product authenticity and potential presence of adulterations. Regulatory measures and transparency of product labeling becomes crucial to ensure consumer's safety and product integrity. The aims of the research were the development and validation of a GC-MS method for the simultaneous determination of terpene compounds for the purposes of quality control of essential oils, as well as quality control of commercially available peppermint essential oils. The GC-MS method was validated in terms of selectivity, precision, accuracy, linearity, detection, and quantification limits. Only one out of the six samples met the pharmacopeial standards of mint essential oil. In one sample, menthol exceeded the declared limit, while menthofuran was not detected in any of the samples. Pulegone met the specified parameters. Analyses revealed discrepancies between products labeling and chemical composition, emphasizing the requirement for the existance of strict guidelines and regular quality control. Lack of information about the botanical origin creates ambiguity, highlighting the necessity for regulatory updates. Non-compliance with regulatory standards indicates the potential presence of adulterated products, highlighting the requirement for development of more detailedproduct labeling guidelines and authentication verification systems.

Key words: peppermint; essential oils; *Mentha x piperita*; GC-MS; quality control

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RESEARCH ON ALLIUM SPECIES AS SOURCES OF BIOLOGICALLY ACTIVE SECONDARY METABOLITES

Blagoje Prpa[1,2*], Milica Rat[3], Katarina Bijelić[1,2], Maja Hil[1,2], Katarina Jeremić[1], Nebojša Kladar[1,2]

[1]University of Novi Sad, Faculty of Medicine, Department of Pharmacy, HajdukVeljkova 3, 21000 Novi Sad, Republic of Serbia
[2]University of Novi Sad, Faculty of Medicine, Center for Medical and Pharmaceutical Investigations and Quality Control, HajdukVeljkova 3, 21000 Novi Sad, Republic of Serbia
[3]University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, TrgDositejaObradovića 2, 21000 Novi Sad, Republic of Serbia

*blagoje.prpa@mf.uns.ac.rs

The genus *Allium* represents a group of taxa highly diverse in term of secondary metabolitespresence, such asphenolics, organosulfur compounds, organoselenium compounds, and terpenoidcompounds. Numerous garlic species growing wild have shown significant biological effects, such as antioxidant, anticarcinogenic, anti-inflammatory and other. The antimicrobial activity of these plants' secondary metabolites is of particular importance, recognized in bothethnopharmacologyand conventional medicine. As the phenomenon of antimicrobial resistance to conventional antibiotics is significantly progressing, the discovery and utilization of alternative antimicrobial agents become particularly important. Given that the genus *Allium* is rich in such compounds, understanding the chemotaxonomic characteristics of this group is crucial for identifying species thatcould serve as alternatives to available antimicrobial agents. Based on the content of organosulfur compounds, the genus *Allium* can be divided into three groups (methiin type, alliin type, and propiin/isoalliin type). Furthermore, in relation to the abundanceand mutual share ratio of chemotaxonomicmarkers (of which isoalliin, alliin, methiin, and propiin are particularly prominent), it is possible to distinguish six chemotypes among the three aforementioned groups. Flavonoidsare another important class of biologically active secondary metabolites present in *Allium* sp., among which quercetin and kaempferol emerge as the most significant chemotaxonomic characters. The content of plant phenolics and organosulfur compounds supports the cladistics obtained through the application of molecular, karyological, anatomical, and morphological characters, emphasizing the importanceof chemotaxonomy for understanding phylogenetic relationships within the genus *Allium*, as well as a tool for more efficient search of sources of biologically active compounds.

Key words: *Allium*, chemotaxonomy, organosulfur compounds, flavonoids

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ANTIBIOTIC USAGE DURING PREGNANCY IN THE BITCHES: HOW BAD IS IT?

Ivan Galić[1*], Ivan Stančić[1], Jelena Apić[2], Vuk Vračar[1], Ivan Butković[3], Stevan Rodić[2], Zorana Kovačević[1]

[1]University of Novi Sad, Faculty of Agriculture, Department for Veterinary Medicine, Novi Sad, Serbia
[2]Scientific Veterinary Institute "Novi Sad", Novi Sad, Serbia
[3]University of Zagreb, Faculty of Veterinary Medicine, Clinic for Reproduction and Obstetrics, Zagreb, Croatia

*ivan.galic@polj.uns.ac.rs

Due to the possible harmful effects on the fetus, the use of drugs during pregnancy is a particular therapeutic challenge. When deciding on a particular drug, the veterinarian always considers its safety during pregnancy. When using a particular drug in a particular situation, it is important to weigh up the balance of benefits and risks at each stage. Sometimes the benefits outweigh the risks, and drugs that are associated with risks can be used to preserve the health of the mother. The use of pharmacological agents in the pregnant females possess a major clinical challenge due to the marked physiological changes that can alter the pharmacokinetics of drugs and the potential effects on the fetus. Fetal development, decreased digestive rate, increased and redirected blood flow, and increased renal function can alter the way the body processes a drug. Some antibiotics can be administered during pregnancy, but they carry the risk of side effects (malformations or death of the puppies). Pregnancy in the bitches lasts around 62- 65 days, and careful monitoring must be carried out during this time if treatment with drugs has been carried out. The first trimester (6th to 20th day of gestation) is the critical period for embryotoxicity. Drug administration should be avoided during this period if possible, especially drugs known to cause pregnancy loss. The first classification of the teratogenic risk of veterinary drugs most commonly used in the treatment of dogs was made more than thirty years ago. The beta-lactams are the first choice for treatment of infections during pregnancy. Macrolides are generally used clinically in patients who are allergic to beta-lactams. Antibiotics that have been shown to be safe for use in pregnancy include beta-lactam antibiotics (penicillin G, ampicillin, amoxicillin, amoxicillin-clavulan, carbenicillin, ticarcillin, and cephalosporins), macrolides and lincosamides (clindamycin, erythromycin and lincomycin). The general tendency to avoid the use of drugs in pregnant women and animals means that there is little previous experience to serve as a frame of reference. On the other hand, breeders and dog owners must be aware that excessive use of antibiotics without a prior antibiogram does not reduce the neonatal mortality of puppies, but on the contrary increases the risk of antibiotic-resistant strains of bacteria. The aim of this article is to give a brief overview of what we know about the use of antibacterial drugs in bitches during pregnancy.

Key words: reproduction, dog, drugs

PIG PRODUCTION WITHOUT ANTIBIOTIC USE? PRACTICAL EXPERIENCES WITH PROBIOTICS CULTURES, IgY AND ORGANIC ACIDS APPLICATION

Damir Rimac[1*], Davor Vasiljević[2], Đuro Čalić[2]

[1]Sano – The Modern Animal Nutrition, Popovača, Croatia
[2]Sano – The Modern Animal Nutrition, Novi Sad, Serbia
*d.r@sano.hr

*Pig breeding expert for Southeast Europe

Reducing the use of antibiotics (AMU - Antimicrobial usage) is the key focus of modern pig production, and reducing antimicrobial resistance (AMR) is a priority of the European Commission according to the "European Action Plan "One Health" to combat AMR" (European Parliament resolution of September 13, 2018 on a European "One Health" Action Plan against Antimicrobial Resistance). Reducing the use of antibiotics in livestock production is also defined by the latest document Green Plan 2050 - GREAT DEAL Europe, https://ec.europa.eu/info/sites/info/files/european-green-deal-communication_en.pdf. The use and abuse of antibiotics in animal feed has led to problems with drug residues in animal products and increased bacterial resistance. The use of different lactic acid bacteria (LAB) such as *Enterococcus faecium* or *Bacillus lichenioformis* or *Bacillus subtilis* in combination with specific immunoglobulins from chicken eggs (IgY) can significantly contribute to strengthening the general health condition of small piglets and their resistance to gastrointestinal problems associated with diarrhea. The application of 4 ml ***Piglobin***® product within 6 h after birth and 2 ml more second day significantly reduced the occurrence of neonatal diarrhea in the two analyzed groups of piglets ($p < 0.06$) by 28%, while the consumption of antibiotics in suckling piglets was also significantly reduced ($p < 0.07$) by 40%. At the same time, piglet deaths were reduced by 21.16%, but were not statistically significant ($p > 0.05$). The use of milk replacers enriched with LAB as well as pre-starters enriched with LAB and mixture of organic acids also modulates the microbiota of the gastrointestinal tract (GIT) of small piglets with the expression of better general health, better daily gains, and reduced AMU. By applying a mixture of different organic acids (OA) that are added through food in granular form (formic C1:0 or CH_2O, butyric C4:0 or $C_4H_8O_2$, lauric acid C12:0 or $C_{12}H_24O_2$) or through water in liquid form via medicator (propionic C3:0 or $C_3H_6O_2$ and formic acid – C1:0 or CH_2O) it is possible to achieve very good results in pig production technology without the use of antibiotics due to the modulation of the microbiota of GIT. Exceptionally good production results were achieved by applying a 0.3% mixture of organic acids (***Acidosan***®) through water (via medicator) in piglets after the weaning phase (reducing on average 82% cases of edema disease and colibacillosis diarrhea). In fattening piglets after importation and during feed change (after 70 kg of body weight), losses were on average 58% lower. Most deaths after 70 kg were related to the *peracute* form of *Clostridium perfrigens and animal quality.* Also, when piglets arrived for fattening, instead of antibiotics, they were given a mixture of organic acids through water. Recommended amounts of organic acids in feed for preventive action against digestive disorders and reduction of AMU are within 0.2 - 0.8% depending on the type of acid, as well as on the category of pigs for which the feed is prepared.

Key words: Probiotics, IgY, organic acids, AMU reduction, health status

CONSUMPTION OF ANTIBIOTICS IN SERBIA IN 2021 AND THEIR IMPACT ON THE ENVIRONMENT

Dušan Prodanović[1*], Zorana Kovačević[2], Olga Horvat[1]

[1]Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia

[2]Faculty of Agriculture, Department of Veterinary Medicine, University of Novi Sad, Novi Sad, Serbia

*dusan.prodanovic@mf.uns.ac.rs

Due to the improvement of the quality of life of the population, the daily advancement of medicine, the improvement of therapeutic regimens, as well as the extension of the average life spantogether with the extension of therapy of chronic patients, increased consumption of all drugs is observed. In addition to increasing resistance, irrational and excessive use of antibiotics also has a negative environmental impact. Due to the way medicines and drug waste are disposed of, their greatest adverse effect is observed in water and aquatic ecosystems. This research aims to analyze the consumption of antibiotics in Serbia and their impact on the environment. Data on antibiotic consumption have been collected from the publication of the Agency for Medicinal Products and Medical Devices of Serbia (ALIMS) for the year 2021. The consumption of drugs is expressed in defined daily doses (DDD) and the total annual mass of the antibiotic consumed. Environmental impact assessment of various antibiotics was carried out by Environmental Hazards (PBT), which takes into account: the presence - resistance of the degradation substance in the aquatic environment; bioaccumulation - the accumulation of substances in the adipose tissue of aquatic organisms; and toxicity, the potential of a substance to poison the aquatic organism. For each of these three parameters, a number from 1 to 3 is assigned, so that the PBT values for all substances range from 0 to 9. For some of the substances, there is still no formed PBT score, but this does not mean that these substances are not harmful to the environment and that additional studies need to be carried out. In 2021, looking at the number of DDDs for each of the antibiotics, it is noted that azithromycin (11375052 DDD), levofloxacin (10676584 DDD), and amoxicillin (8406562 DDD) were the most widely used drugs and that the total consumption of these three antibiotics is higher than all other antibiotics combined. However, regarding the mass of antibiotics consumed in 2021, we notice that amoxicillin with 12.6t had the highest consumption, with cephalexin (8.8t) and ceftriaxone (5.6t) in second and third place, respectively. Almost all antibiotics we observed have PBT 6, except for ciprofloxacin, whose PBT is 9, and whose consumption in the observed period was 3906432 DDD or 3.9t. Excessive use of antibiotics, especially if it is limited to only a few drugs, in addition to increasing resistance, also significantly increases their harmfulness to the environment. Particular attention should be paid to drugs with high PBT, such as ciprofloxacin, which can severe negative impact on the aquatic ecosystem and the organisms that live in it.

Key words: antibiotics, consumption, PBT, amoxicillin, azithromycin, ciprofloxacin

HUMAN-APPROVED ANTIBIOTICS IN THE VETERINARY PRACTICE: INSIGHTS FROM PET OWNER INTERVIEWS

Dragana Tomanić[1*], Olga Horvat[2], Ivan Galić[1], Zoran Ružić[1], Zorana Kovačević[1]

[1]Department of Veterinary Medicine, Faculty of Agriculture, University of Novi Sad, TrgDositejaObradovica 8, 21 000 Novi Sad, Serbia
[2]Department of Farmacology and Toxicology, Faculty of Medicine, University of Novi Sad, HajdukVeljkova 3, 21000 Novi Sad, Serbia
*tomanicd@live.com

Market share of veterinary drugs globally comprises only 4-5% and it is even smaller in Serbia. Hence, drugs approved for use in humans are frequently used for treating infections in the companion animals, especially in dogs and cats. This use is known as “off label” use and is defined as use of drug outside its officially approved indications. Additionally, the widespread use of human drugs in the companion animal practice may be due to the factors such as more suitable pharmaceutical formulation of drug, strength, package size etc. Therefore, the aim of this research is to determine what type of human approved antibiotics have been prescribed for companion animals in veterinary practice in Serbia. The survey was conducted through face-to-face interviews with pet owners in Novi Sad, Serbia from December 2023 to February 2024, during their visit to the different veterinary clinics. The study included 300 respondents, with 28.3% of male participants (whilethe majority of respondents were female (71.7%). The findings reveal that dogs comprise the majority, accounting for two-thirds (66.7%) of the pets, followed by cats (22.8%), with all other animal species representing only 10.5%. When asked whether their pets had received medication in the past 5 years, the majority of respondents (70.7%) confirmed that their pets had been prescribed medication. Concerning the type of medication administered, in almost half of the pets (47.9%) owners reported human-approved drugs have been prescribed, while 41.6% said that their pets have not received such medication. Additionally, 10.5% of respondents expressed uncertainty regarding their pets' medication status. Antibiotics were the most commonly prescribed drugs for these animals, representing 55% of all human approved drugs. These antibiotics belong to following classes: beta-lactams (30.2%), nitroimidazoles (27.1%), tetracyclines (21.87%), aminoglycosides (17.7%) and lincosamides (3.13%). Regarding the active ingredients, metronidazole (27.1%) was the most common prescribed, followed by doxycycline (21.87%), amoxicillin with clavulanic acid (15.62%) and ceftriaxone (3.12%). Regarding administration route, most of them were for systemic (oral or parenteral), while minority were applied topically (skin, eye, ear). Beta-lactams, lincosamides, tetracyclines, and metronidazole were always used systemically, whereas aminoglycosides (tobramycin and gentamicin) were only used topically. The most common administration route was oral (49.5%), followed by topical (30.9%) and parenteral (6.7%). In conclusion, this research sheds light on the prevalent trend of "off-label" use of human-approved antibiotics in Serbian veterinary practice, in the treatment of companion animals, highlighting a significant reliance on such pharmaceuticals. Furthermore, the findings underscore the urgent need for regulatory oversight and guidelines regarding the use of human-approved antibiotics in veterinary medicine. Clear protocols and education initiatives are imperative to ensure the responsible and judicious use of antibiotics, safeguarding animal welfare, and mitigating the risk of antimicrobial resistance. Additionally, future research should delve deeper into the motivations behind the preference for human drugs, exploring factors such as cost-effectiveness, accessibility, and perceived efficacy. Ultimately, addressing these issues will contribute to the development of sustainable veterinary healthcare practices that prioritize both animal health and public health concerns.

Key words: antimicrobials, off label use, human drugs, pets, questionnaire
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IN VITRO EFFICACY OF SELECTED ESSENTIAL OILS AGAINST *ESCHERICHIA COLI* INDUCED BOVINE MASTITIS

Dragana Tomanić[1], Miodrag Radinović[1], Ivana Čabarkapa[2], Ivan Stančić[1], Nebojša Kladar[3,4], Jovan Stanojević[1], Zorana Kovačević[1]

[1]Department of Veterinary Medicine, Faculty of Agriculture, University of Novi Sad, Serbia
[2]Institute of Food Technology, University of Novi Sad, Serbia
[3]Center for Medical and Pharmaceutical Investigations and Quality Control, Department of Pharmacy, Faculty of Medicine, University of Novi Sad, Serbia
[4]Department of Pharmacy, Faculty of Medicine, University of Novi Sad, Serbia

*tomanicd@live.com

Bovine mastitis is a complex disease involving interactions between animals, their environment, and microorganisms, with a wide range of microorganisms having the greatest importance. Among the various microorganisms involved, *Escherichia coli* is recognized as the most common gram-negative pathogen causing clinical mastitis in cows. It is stated that *E. coli* has the potential to become resistant to almost all antibiotics due to the irrational use of antibiotics in the treatment of cow mastitis. Consequently, there is a pressing need for alternative therapeutic strategies. Essential oils (EOs) have emerged as a promising option due to their safety profile and pharmacological properties, making them potential replacements for conventional antibiotic therapy. Therefore, the aim of this study was to evaluate the *in vitro* efficacy of selected EOs against *E. coli* isolates. Isolates were obtained from milk samples taken from cows with mastitis on two dairy farms in Serbia and were identified using standard microbiological methods. Antibacterial activity of EOs was evaluated using the microdilution technique. Among 70 milk samples, 15 isolates (21,4%) of *E. coli* were obtained. EOs of common thyme (*Thymus vulgaris* L., Lamiaceae), wild thyme (*Thymusserpyllum* L., Lamiaceae), oregano (*Origanumvulgare* L., Lamiaceae), mountain savory (*Saturejamontana* L., Lamiaceae) peppermint (*Mentha x piperita* L., Lamiaceae) and lemon balm (*Melissa officinalis* L., Lamiaceae) were used in the research. The selected EOs are commercially available on the market of the Republic of Serbia, produced by a certified manufacturer. Antimicrobial potential is expressed as minimal inhibitory (MIC) and minimal bactericidal concentration (MBC). MIC for the tested EOs ranged from 0.78 to >100 mg/mL, while MBC ranged from 6.25 to >100 mg/mL. The obtained MIC and MBC values of tested EOs indicate that oregano EO had the highest antimicrobial potential against *E. coli* isolates (MIC/MBC=0.78/1.56 mg/mL), while peppermint and lemon balm EO had the lowest antimicrobial potential (MIC/MBC=>100/>100 mg/mL). In conclusion, thisstudy highlights the promising antimicrobial activity of selected EOs, particularly oregano EO, against *E. coli* strains associated with bovine mastitis. These findings suggest the potential of EOs as alternative therapeutic candidates for mastitis, emphasizing the need for further research to evaluate their*in vivo*clinical efficiency.

Key words: antibiotics, dairy cows, essential oils, mastitis

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RHIZOSPHERIC *BACILLUS* SPP. AS AN ALTERNATIVE TO CHEMICALCONTROL OF*BOTRYTIS CINEREA* ON TOMATO

Vasiljka Karačić[1*], Dragana Miljaković[2], Milan Ivanović[1]

[1]University of Belgrade - Faculty of Agriculture, Nemanjina 6, 11080 Belgrade
[2]Institute for Agriculture and Vegetables, Maksima Gorkog 30, 21101 Novi Sad

*vasiljka.dragic@gmail.com

Botrytis cinerea is one of the most destructive fungal pathogens affecting over 200 plant hosts, including important crop species such as tomato (*Solanum lycopersicum* L.). This fungus causes gray mold of tomato, affecting the yield in the open field, greenhouses, but also during the transportation and storage of the product. *B. cinerea*is difficult to control because it can survive as mycelia, conidia or sclerotia for long periods, while numeroushosts serve as the source of inoculum. Control of tomato gray mold involves crop rotation, sanitation and improved growing conditions, but still largely relies on repeated application of fungicides. Excessive use of fungicides has led to development of *B. cinerea*resistance to various groups of fungicide. Resistance to carbendazim and procymidone in *B. cinerea*isolates originating from tomato has been confirmed in Argentina, while resistance to difenoconazole has been reported in China and resistance to carbendazim and cyprodinil was foung in Greece. Therefore, it is classified as high risk pathogenfor the development of resistance by Fungicides Resistance Action Committee (FRAC). Occurrence of resistance coupled with public concern on fungicide residues in vegetables created anurgent need to find an alternative method to control *B. cinerea*. One approach is the utilization of bacteria from the genus *Bacillus*. *Bacillus* spp. represent a heterogeneous group of bacteria, which are dominant in the soil and the rhizosphere of plants. Theybelongto Gram-positive bacteria with rod-shaped cells andability to produce endosporesin unfavorable conditions for growth. *Bacillus* species have numerous positive effect on plants, such as ability to increase the availability of nutrients in the soil, stimulate plant growth and control plant pathogens. These bacteria protect plants from pathogens by producing antimicrobial compounds (AMCs), competing for niches and nutrients, and inducing systemic resistance. In our experiment, two-month-old tomato plants (BBCH 59, variety Novosadski jabučar), were treated foliarly once a week for three consecutive weeks with either 30 or 55 ml of bacterial suspension of *B. amyloliquefaciens* isolate Bac 28.3. One day after the last treatment, all plants were spray-inoculated with conidial suspension (10^5 conidia ml^{-1}) of *B. cinerea* isolate 463-19. Systemic fungicide Switch 62.5 WG (Syngenta) was used at recomended concentration (0.23%) as positive control, and sterile distilled water (SDV)as negative control. Appearance of symptoms on treated plants was monitored daily, and Disease severity (DS in %) was assessed 7, 10 and 14 days after inoculation. With the 30 ml-dose, the highest efficiency in suppressing *B. cinerea* on tomato plants was obtained with isolate Bac 28.3 (DS=63.88%), compared to SDV (DS=86.1%) and the fungicide Switch 62.5 WG (DS=69.44%).Similar results were obtained with a dose of 55 ml: DS of 59.72% when treated with isolate Bac 28.3, which was statistically significant compared to 86.10% (water), but not statistically significant compared to 69.44% (Switch).Subsequent PCR analysis of Bac 28.3 isolate revealed presence of genes for two cyclic lipopeptides: bacillomycin D and iturin A, indicating the intrinsic trait underlying the inhibitory effect on *B. cinerea*.

Key words: fungicides, residues, resistance, gray mold of tomato, disease severity

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THE CONTROL OF EFFLUENT WATER FROM RECIRCULATING AQUACULTURE SYSTEM (RAS): REGULATORY COMPLIANCE IN SERBIA

Miroslav Urošević[1*], Sara Čer[1], Beata Abramowicz[2]

[1]University of Novi Sad, Faculty of Agriculture, Dep. of Animal Science Novi Sad, Serbia
[2]University of Life Sciences in Lublin, Department and Clinic of Animal Internal Diseases, Poland

*miroslav.urosevic@stocarstvo.edu.rs

The necessity of aquatic farming has risen as a consequence of an increasing world population because fish catch production has reached its limits. New technologies, such as land-based production in Recirculating Aquaculture Systems (RAS) are creating opportunities for the growth of high-value species closer to the market and allow for more environmentally sustainable production and reduction in carbon footprint related to fish transport. In RAS water coming from fish tanks is cleaned and recycled allowing for a 99% reduction in the amount of water used for production. At the same time nutrients from the effluent water are removed minimizing the eutrophication effect of production on the surrounding environment. All European Union countries have strictly regulated technical requirements for RAS use. I.e. in the Water Law Act in Austria (2004) for discharging aqueous emissions from an RAS following Annual production capacity, the emission limits of different substances must be prescribed. In any case, the following substances may not be discharged: Chloramphenicol; Furazolidone; Dimetridazole; Malachite green - oxalate; and Nitrofurans. Although the field of fisheries is up to some point regulated by the contemporary regulatory framework in Serbia, still concerning RAS or "closed aquaculture facilities" in comparison to EU regulations there is no equally precise act. Additionally, the lack of many important provisions, present in EU legislation, is obvious. In the Animal Husbandry Law of Serbia (2009), RAS or "closed aquaculture facilities" are not precisely defined, nor mentioned, but only can be subjected as "other facilities". In light of modern requirements for fish production and environmental protection, defining and incorporating RAS into the regulatory framework represents a necessity. Unfortunately, for now, there is no regulation on the limit values of chemical and biological substances/substances from RAS in Serbia. This means that it is practically possible to discharge wastewater into rivers, canals, or onto agricultural land, without any control over the presence of, for example, antibiotics or other drugs. This is also supported by the fact that RAS systems in Serbia can be registered as outdoor ponds (lakes), which such systems are certainly not. We advise harmonization of the Serbian legislation to the European standards regarding the precise definition of RAS in the frame of animal production, veterinary and health issues, as well as conditions connected with strict environmental protection rules. It especially refers to the establishment of precise instructions in the future rulebook on RAS related to waste water by wastewater.

Key words: Closed aquaculture system, European Union, waste water, antibiotic residue, regulation

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PREVALENCE OF ANTIMICROBIAL RESISTANT *ESCHERICHIA COLI* AND *KLEBSIELLA PNEUMONIAE* FROM SHEEP AND GOATS IN VOJVODINA PROVINCE

Vesna Lalošević[1], Danijela Petrovic[2], Gordana Kozoderović[2], Vuk Vračar[1*]

[1]University of Novi Sad, Faculty of Agriculture, Department of Veterinary Medicine, Novi Sad, Serbia
[2]University of Novi Sad, Faculty of Education in Sombor, Department of Natural Sciences and Management in Education, Sombor, Serbia

*vuk.vracar@polj.uns.ac.rs

The presence of antibiotic-resistant bacteria in sheep and goats is considered a serious burden to public health and impacts the economy given the fact that small ruminants comprise the highest population among all livestock species worldwide. In this view, we examined the incidence of antimicrobial resistance among commensal *Escherichia coli* and *Klebsiella pneumoniae* isolated from 42 sheep and 20 goats in Vojvodina Province. Fecal samples of 62 healthy animals were cultivated on standard Endo agar plates and 30 isolates of E. *coli* from sheep and 15 from goats were identified based on classical biochemical tests. Also, in each group of animals one isolate of *Klebsiella pneumoniae* was found. Resistance among *E. coli* isolates to the most frequently use antibiotics was done by standard disc diffusion method. The prevalence of resistance to one or more antimicrobials in all *E. coli* isolates from small ruminants was 44,4% (20/45). Resistance to antibiotics was found in 14 out of 30 *E. coli* isolates from sheep fecal samples. More than a half of those, 57,1% (8/14), was resistant to two antibiotics, ampicillin and amoxicillin/clavulanic acid. Three isolates were resistant to ampicillin only, 21,4% (3/14), one to cefuroxime, 7,14% (1/14), gentamicin and sulfometoksazole/trimethoprim, each. *E. coli* from goats showed that 40,0% (6/15) of isolates were resistant to only one antimicrobial agent - ampicillin. *Klebsiella pneumoniae* isolated from sheep was multiresistant to four antibiotics (ampicillin, amoxycillin/clavulanic acid, gentamicin and amikacin), and that from goat was resistant to three antibiotics (ampicillin, amoxycillin/clavulanic acid and gentamicin). Based on the fact that resistance to colistin is often found in intestinal bacteria of small ruminants, we examined extracted total genomic DNA from 31 randomly chosen fecal samples for the presence of *mcr-1*, *2*, *3*, *4*, *5* genes as a marker of colistin resistance by the multiplex PCR. However, none of the samples confirmed the presence of particular *mcr* genes. The data indicate that antimicrobial resistance in *E. coli* and *Klebsiella pneumoniae* of different animal origin could be due to a broad range of acquired genes. These results reveal that close monitoring of the antimicrobial resistance of food animal production is essential and that it could reduce the potential public health risks in both veterinary and human medicine.

Key words: sheep, goat, antimicrobial resistance

INSIGHTS INTO ANTIBIOTIC UTILISATION. PILOT STUDY FROM AN EMERGENCY DEPARTMENT

Erika Papfalvi[1,4*], Róbert Nacsa[1], Mária Matuz[1], Roxána Ruzsa[1], Helga Hambalek[1], Edit Hajdú[3], Zoltán Pető[4], Péter Erdélyi[4], Gyöngyvér Soós[1], Ria Benkő[1,3,4]

[1]Clinical Pharmacy Institute, Faculty of Pharmacy, University of Szeged, Szeged, Hungary;
[2]Central Pharmacy, Albert Szent Györgyi Medical Center, University of Szeged, Szeged, Hungary
[3]Department of Infectious Diseases, University of Szeged, Szeged, Hungary
[4]Department of Emergency Medicine, University of Szeged, Szeged, Hungary

*papfalvierika@gmail.com

The global challenge of antimicrobial resistance (AMR) necessitates a strategic approach to excessive antibiotic use to control its negative impacts. In the context of limited data on antibiotic use in Emergency Departments (ED), this study was meant to analyze antibiotic initiation patterns, documentation rates for prescribing antibiotics, and the frequency of microbiological sampling. Conducted at a tertiary care medical center's ED, this retrospective observational field study involved 2140 adult ED patients over 24 randomly selected days. Medical records were reviewed to identify cases where antibiotics were prescribed and to analyze patient and antibiotic treatment-related data. We found that antibiotics were initiated in 231 patients, constituting 10.8% (95 % CI: 9.55%-12.18%) of all ED presentations. The majority of antibiotic treatments (131) were commenced as parenteral therapy in the ED, 17 of these patients were discharged with antibiotic prescription later on. 100 patients were given only oral antibiotic courses. Common indications for antibiotics included urinary tract and intraabdominal infections. The most commonly prescribed antibiotics were intravenous ceftriaxone and oral co-amoxiclav. In addition, a substantial portion of patients were above the age of 65 years (62%) and 52% of patients presenting a high comorbidity index (Charlson-Comorbidity index score above 5 or more). In 23.4% neither the exact indication, nor related information about the antibiotic initiation was documented, and insufficiencies were found in documentation in further 20%. Microbiological sampling was performed in 48% of these patients. The study highlights that the prevalent initiation of antibiotics in the ED demands the imperative to address more attention on quality issues in antibiotic use.

Key words: antibiotic stewardship, antimicrobial resistance, emergency department, rational antibiotic use

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DRUG-DRUG INTERACTIONS OF THE RESERVE ANTIBIOTICS

Bojana Božić Cvijan[1*], Miljana Labović[2], Marija Kukurić[2], Milica Bajčetić[1,3]

[1]Department of Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Belgrade, P.O. Box 38, 11000 Belgrade, Serbia
[2]Faculty of Medicine, University of Belgrade, 11000 Belgrade, Serbia
[3]Clinical Pharmacology Unit, University Children's Hospital, 11000 Belgrade, Serbia

*bojanabozic87@gmail.com

Drug interactions often cause side effects, especially in children, elderly and/or patients with chronic diseases. Antibiotics are among the most commonly used drugs, so potential impact of antibiotic-drug interactions on the ultimate outcome of therapy may be of great clinical value. Having in mind that antibiotic-drug interactions can lead to development of antimicrobial resistance (AMR), their identification is especially important for reserve antibiotics. The reserve group, so called "last resort" includes antibiotics that should be saved for the infections caused by multidrug-resistant microorganisms. Representatives of reserve antibiotics are ceftazidime-avibactam, ceftolozane-tazobactam, ceftarolinefosamil, cefiderocol, ceftobiprole-medocaril, meropenem-vaborbactam, imipenem-cilastatin/relebactam, faropenem, aztreonam, carumonam, oritavancin, dalbavancin, telavancin, daptomycin, fosfomycinintravenous (IV), dalfopristin/quinupristin, linezolid, tedizolid, lefamulin, minocycline IV, omadacycline, tigecycline, eravacycline, iclaprim, colistin oral and IV, polymyxin B oral and IV, plazomicin. The aim of this study is to analyze the drug-drug interaction potential of reserve antibiotics. The highest potential for antibiotic-drug interactions was identified with linezolid, colistin, dalfopristin/quinupristin, lefamulin and oritavancin. Special caution should be paid to concomitant administration of ceftazidime-avibactam, telavancin, colistin, polymyxin B, plazomicin with drugs that have nephrotoxic potential due to possibility of more severe renal impairment. Exceptional wariness is required when combining drugs with reserve antibiotics with limited drug-drug interactions information such as plazomicin, carumonam, iclaprim. Having in mind that antibiotic-drug interaction can lead to the changed antimicrobial efficiency and/or safety of the therapy, the antibiotic choice has to be based on data regarding interaction potential. Continuous education of clinical staff regarding the choice of antibiotics based on their interaction potential and optimizing the antibiotic dose may significantly improve pharmacotherapy and decrease the risk for AMR.

Key words: antibiotic, drug-drug interactions, reserve antibiotics, AMR

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PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PROFILES OF THE CONJUNCTIVAL BACTERIAL ISOLATES FROM THE CANINE EYES

Nadežda Tešin[1*], Ivan Stančić[1], Bojan Toholj[1], Dragana Božić[2], Jovan Spasojević[1], Ivan Galić[1], Dragana Tomanić[1], Zorana Kovačević[1]

[1]University of Novi Sad, Faculty of Agriculture, Department of Veterinary Medicine, Novi Sad, Serbia

[2]University of Belgrade, Faculty of Pharmacy, Department of Microbiology and Immunology, Belgrade, Serbia

*nadezda.tesin@polj.edu.rs

Bacterial conjunctivitis represents one of the most common ocular pathology that affects canine population. While different types of bacteria can be involved in the disease development, infection often can be spread to the surrounding tissues. Due to increased antimicrobial resistance (AMR) and number of resistance strains in the veterinary ophthalmology, the objective of this study was to evaluate the occurrence and the antimicrobial susceptibility profiles of bacteria isolated from the eyes of dogs with diagnosed conjunctivitis. This research and sampling were performed at the Veterinary Clinic for Small Animals, Faculty of Agriculture, University of Novi Sad. Swabs were sent to the laboratory to be microbiologically tested. After incubation and identification, it was found that out of a total of 26 samples, 20 (77%) were positive for the presence of pathogens. All isolated bacteria belonged to staphylococcal and streptococcal genera, while *Staphylococcus pseudintermedius* was the most commonly isolated bacterium (38%). Fluoroquinolones were the most efficient antimicrobials against staphylococci, whereas against streptococci there were amoxicillin with clavulanic acid and ceftriaxone. It could be concluded that the identification and antibiotic susceptibility testing for pathogen associated to conjunctivitis in the different areas is required in order to prevent antimicrobial overuse and mitigate AMR identifying local habits of using certain antibiotics.

Key words: antimicrobial resistance, antimicrobial susceptibilities, conjunctivitis, dog, eye

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BACTERIAL CONTAMINATION OF BOAR SPERM

Stevan Rodić[1], Ivan Stančić[2], Jelena Apić[1], Tomislav Barna[1], Dalibor Todorović[1], Igor Stojanov[1], Ivan Galić[2]

[1]Scientific Veterinary Institute "Novi Sad", Rumenački put 20, 21000 Novi Sad
[2]Department for Veterinary Medicine, Faculty of agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad

*stevan.r@niv.ns.ac.rs

Sperm collection is not a sterile process, no matter how hygienic conditions are maintained at a high level. This is the reason whyubiqitousbacteria are important for veterinary and human medicine. The concentration of bacteria found in spermis very important. The natural way of mating does not pose a problem when bacteria are present in the sperm. Immediately after ejaculation, the entire amount of sperm is inserted into the female reproductive tract, which has its own antimicrobial factors and protection mechanisms. The time from the moment of ejaculation to fertilizationis short, therefore the bacteria do not have time to multiply. It is a much bigger problem when the sperm is diluted and divided into doses for artificial insemination, because then the bacteria have time to multiply. This has beenprevented by the addition of antibiotics to the diluents. Bacteria are problem for intensive pig production, because artificial insemination is necessary to maintain this type of production, due to the fact that artificial insemination can produce a much larger number of offspring than natural insemination and the process is much faster. The biggest problem is the period of storage of doses from moment of ejaculation to the moment of insemination, because sperm is an ideal medium for the development of bacteria. Numerous mechanisms by which bacteria can have impact on spermatozoa have been described. Antimicrobial resistance of bacteria representsa real danger, not only when it comes to reproduction, but also in many other areas such as animal health, nutrition, quality of life, etc.

Key words: Sperm, bacteria, antimicrobial drugs, boar, insemination.

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A REVIEW OF THE ALTERNATIVE METHODS FOR TREATMENT OF BOVINE MASTITIS

Jovan Stanojević[1*], Miodrag Radinović[1], Marija Pajić[1], Zorana Kovačević[1], Dragana Tomanić[1], Nadežda Tešin[1]

[1]University of Novi Sad, Faculty of Agriculture, Department of Veterinary medicine, Novi Sad, Serbia

*jovan2912@email.com

Cow mastitis is one of the most important diseases of high producing cows in modern milk production. In dairy cows, antibiotics are the most use for treatment of mastitis. Conventional treatments with antibiotic could leave antibiotic residues in the milk and lead to development of resistant bacteria. Especially, the uncontrolled use of antibiotics without determing the antibiogram in mastitis therapy is an important factor in the spread of antimicrobial resistance in dairy cattle. Besides, residues of antibiotics in milk and other animal product can causing a public health problem, including allergic reaction, immunopathological effects, carcinogenicity, mutagenicity, nephropathy, hepatotoxicity, reproductive disorders and anaphylactic shock in humans. For this reason the use of alternative methods for treatment of bovine mastitis such as fytotherapy, probiotics, cytokines, bacteriophages and vaccination are very important in decrease of use antibiotics and consequently reduce of antimicrobial resistance.

Key words: cow, mastitis, alternative therapy

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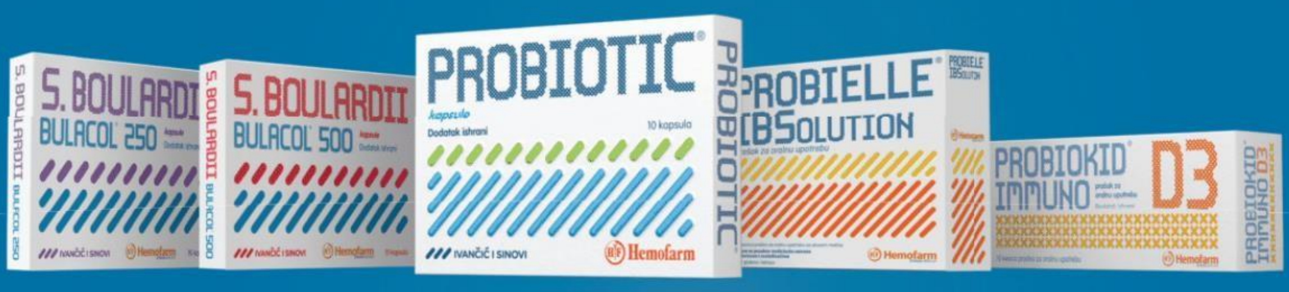

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