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ONE HEALTH: CHALLENGES FOR FOOD SAFETY

Abstracts

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ANTIMICROBIAL RESISTANCE: OUR ONE HEALTH CHALLENGE

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Antimicrobial resistance (AMR) is one of the current top global health issues. Often, it is simplified to the version “resistance to antibiotics”, and therefore associated by many only to human health. In reality, its dimensions are much broader, as all antibiotics are antimicrobials, but not all antimicrobials are antibiotics. Antimicrobials are used not only in human medicine but also in animals and plants/crops, with a spill over to the environment, making it a perfect illustration of a One Health issue.

In particular after the adoption of the Global Action Plan (GAP) on AMR in 2016, a multitude of initiatives have been implemented at different levels, many of them focusing on: i) raising awareness on AMR; ii) data collection; and iii) responsible and prudent antimicrobial use, when needed. AT FAO, the AMR Action Plan is the overall umbrella that covers, for example, the development of the InFARM (International AMR Monitoring System) database and the RENOFARM (Reduce the Need for Antimicrobials on Farms for Sustainable Agrifood Systems Transformation) initiative.

AMR can also be foodborne, and the same can be said the other way around: some of the foodborne infections are caused by antimicrobial resistant microorganisms. The exact dimensions of these phenomena are not quantifiable on a global scale. It is important to emphasize that AMR is also a food safety issue, that threatens future global food security.

The cornerstones to control AMR are: 1) a change in behaviors to preserve antimicrobials as global common goods and 2) an agrifood systems transformation that ensures a sustainable food production.

Keywords: antimicrobial resistance (AMR), antimicrobial use (AMU), food safety, one health.

A ONE HEALTH APPROACH TO PREVENT AND COMBAT CAMPYLOBACTER INFECTIONS

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Human campylobacteriosis occurs predominantly after consumption of contaminated chicken meat. According to a recent EFSA report, a total of 31% of chilled broiler carcasses sampled at slaughterhouses were *Campylobacter*-positive. The potent pathogens cause acute and severe enteritis in humans (even after ingestion of low pathogen doses in the range of hundreds of living bacteria), but are harmless commensals in domestic animals. The asymptomatic colonization in chicken flocks supports the formation of huge pathogen reservoirs. Thus, reduction of *Campylobacter* contamination in the poultry production line is key to the prevention of human campylobacteriosis.

An interdisciplinary One Health approach and a combined effort of all stakeholders are necessary to ultimately reduce the burden of campylobacteriosis cases.

However, numerous studies point out that, at present, a complete elimination of *Campylobacter* species in the (poultry) food chain is not feasible. Thus, the current aim should be to establish intervention strategies to minimize the occurrence of *Campylobacter* spp. in livestock (esp. poultry) and to reduce the quantitative *Campylobacter* burden along the food chain in animals and subsequently in foods. The most effective measures to mitigate *Campylobacter* focus on the primary production stage. Nevertheless, measures applied during slaughter and processing complement the general meat hygiene approaches by reducing faecal contamination during slaughtering and processing and, as a consequence, help to reduce *Campylobacter* in poultry meat. That has to be accompanied by targeted consumer advice and education campaigns to raise the awareness towards *Campylobacter* infections.

Keywords: campylobacter, food safety, zoonotic diseases, one health.

IDENTIFYING AND QUANTIFYING MICROBIAL COMMUNITIES IN SARDINIAN SHEEP CHEESE DAIRIES' PROCESSING ENVIRONMENTS VIA DNA METABARCODING

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Microorganisms in dairy processing facilities pose significant challenges to food safety and quality. Despite stringent hygiene practices in Sardinian sheep milk processing, microbial safety issues persist, often due to cross-contamination during post-sanitization processing stages. Environmental conditions and inadequate cleaning procedures facilitate this contamination, creating specific ecological niches for survival and proliferation of microorganisms, including pathogens like *Listeria monocytogenes*, *Salmonella* spp., *Bacillus cereus*, and *Pseudomonas* spp. Traditional culture-dependent methods offer limited insights, prompting the use of high-throughput metagenomic sequencing for a comprehensive understanding.

This study utilized 16S-based bacterial metabarcoding to characterize microbial communities from 14 Sardinian cheese processing environment dairies, analyzing samples from food and non-food contact surfaces. Understanding the microbial ecology is crucial for effective risk management and product safety. *Halomonas* and Halomonadaceae were predominant taxa. *Listeria monocytogenes*, *Salmonella* spp., *Bacillus cereus*, *Escherichia coli*, *Pseudomonas* spp. resulted ubiquitous, present in all samples at an average abundance of 7.50%.

Overall, this research highlights the importance of advanced microbial analysis techniques for identifying and quantifying microbial communities in dairy processing environments, facilitating targeted interventions to enhance food safety and quality assurance measures in Sardinian cheese production.

Keywords: one health, microbiological monitoring, sanitary status, food security, dairy products.

GENES AND PATHWAYS REVEALED BY *ESCHERICHIA COLI* CHALLENGE OF MILK DERIVED BOVINE MAMMARY EPITHELIAL CELLS

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Mastitis is the most common and the costliest bacterial disease affecting the health of dairy cattle. In the animal and food production, the common reason for the use of antimicrobials in dairy cows is mastitis. Bacteria activate the mammary immune system in variable ways, thereby influencing the severity of the disease. Understanding the molecular mechanisms that activate and regulate the host response would be central to effective prevention of mastitis and breeding of cows more resistant to mastitis. We used primary bovine mammary epithelial cell cultures extracted noninvasively from bovine milk samples to monitor the cellular responses to *Escherichia coli* (a common mastitis pathogen) challenge. Differences in gene expression between control and challenged cells were studied by total RNA-sequencing at two time points post-challenge. In total, 150 and 440 (P_{adj} < 0.05) differentially expressed genes were identified at 3 hours and 24 hours post-challenge, respectively. The differentially expressed genes were mostly upregulated. Our results are in line with known effects of *E. coli* infection, with a strong early inflammatory response mediated by pathogen receptor families. Among the most significantly enriched early KEGG pathways were the TNF signaling pathway, the cytokine-cytokine receptor interaction, and the NF-kappa B signaling pathway. At 24 hours post-challenge, most significantly enriched were the Influenza A, the NOD-like receptor signaling, and the IL-17 signaling pathway. We are continuing the work by studying the gene response of mammary epithelial cells when beneficial lactic acid bacteria are introduced during the mastitis pathogen challenge.

Keywords: *Escherichia coli*, mastitis, bovine, infection, primary bovine mammary epithelial cells, transcriptome.

DETERMINATION OF MICROBIOLOGICAL PARAMETERS, ALGAL BIOTOXINS AND TRACE ELEMENTS IN OYSTERS FROM A MEDITERRANEAN LAGOON

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Pacific oysters (*Crassostrea gigas*) are highly nutritious bivalves representing an important dietary constituent. Due to the growing need for aquaculture differentiation, the interest is increasing in Italian oyster farming. This will require the classification of new productive areas in both coastal lagoons and open waters. The objective of the present investigation was to assess the presence of 16 trace elements (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Rb, Se, Sn, V, Zn), biotoxins (PSTs, LTs -OA, DTXs, YTXs and AZAs, DA), *V. parahaemolyticus*, *Escherichia coli* and *Salmonella* spp. in *C. gigas* from an experimental pilot farm set up in the Calich Lagoon (Sardinia, Italy) in the frame of the EU Interreg program RETRALAGS. Samples of *C. gigas* were collected in winter, spring, summer and autumn of 2019. The EU legal limits for Cd and Pb were never exceeded, the farmed oysters were safe to consumers. The results pointed out a high significant seasonal variation of Cd, Mn, Ni, and V. The highest values were found for Fe and Al in autumn, for Zn in winter and spring. *E. coli* was always present overlapping the EU limits of 230 MPN/100g. *Salmonella* spp. was never detected, and *V. parahaemolyticus* was detected only in summer samples. The potentially toxic microalga *Dinophysis acuminata* was found in the lagoon water without accumulation of biotoxins. Pacific oysters were confirmed as suitable bioindicators of the health status of coastal lagoons. Concentrations of trace elements and microbiological parameters were highly affected by season of collection.

Keywords: mediterranean lagoon, food safety, shellfish, trace elements, biotoxins, bacteria.

NATURAL MOLECULES APPROVED AS FOOD ADDITIVES HAVING DUAL ACTIVITY ON FOODBORNE PATHOGENS

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Xanthohumol (XH) is prenylated chalcone originating in hop (*Humulus lupulus* L) which, due to its large number of extremely strong biological activities, including anticancer and immunomodulating, has been attracting the attention of the scientific public for decades. Due to its strong antibacterial effect on Gram-positive bacteria, as well as its non-toxicity for human organism, xanthohumol, as well as hop extract obtained in the process of supercritical fluid extraction (SFE), have been proposed as additives in meat and meat products to prevent the multiplication and elimination of foodborne pathogens, especially in sausages and especially against *L. monocytogenes*. Similarly, carnosic acid (CA), a phenolic diterpene that originates from sage (*Salvia* spp.), rosemary (*Rosmarinus officinalis*), and oregano (*Oreganum* spp.), with its powerful antioxidant and antibacterial effects, has been added to food for decades, and in 2010, all rosemary extracts (including carnosic acid) were categorized as food additives by the European Commission. Still, mechanisms of antibacterial action of all mentioned molecules/extracts are entirely unknown. However, in our investigations within the project “Molecular and physiological response of foodborne pathogens to selected natural bioactive compounds and development of novel biodegradable polymers with antibacterial activity”, (project number 2019/35/B/NZ9/02774 OPUS 18, NCN), we established that the listed molecules can also have a stimulating effect on the multiplication of some foodborne pathogens, and their biofilms, depending on the cultivation conditions. In this study, some of those results are presented, considering that, in relation to human health, those “unexpected” effects can have possibly dangerous consequences.

Keywords: xanthohumol, hop extract, carnosic acid, foodborne pathogens.

SURVIVAL OF BACTERIAL PATHOGENS IN FOOD PRODUCTION ENVIRONMENTS DUE TO A VIABLE BUT NON-CULTURABLE STATE

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Effective monitoring of microbial pathogens is essential for a successful preventive food safety and hygiene strategy. However, as most monitoring strategies are growth-based, these tests fail to detect pathogenic bacteria that have entered dormancy states such as the viable but non-culturable (VBNC) state. Bacteria that enter the VBNC state lose their ability to grow on standard enrichment media, but remain metabolically active, show a drastically increased tolerance against antimicrobials and can remain infective or completely resuscitate from dormancy. While the ability of bacteria to enter the VBNC state has been known for several decades, their impact on public health and food safety has still been drastically under-researched. Since its first description in 1982, researchers have described the VBNC state for more than 100 different bacterial species, of which 67 are pathogenic bacteria and 35 are foodborne pathogens including *Campylobacter*, *Salmonella*, *Listeria*, *Escherichia*, *Staphylococcus*, *Bacillus* and *Vibrio*. Induction of the VBNC state is a response to either unfavorable environmental conditions, such as changes in temperature, nutrient or water availability, or potentially lethal stress by biocides, antibiotics or physical stress. Food is frequently exposed to such conditions during processing, transportation and storage providing ample opportunities for the induction of VBNC cells, and the ability to enter a viable but non-culturable state is likely connected to persistence in food production environments. In this context, we present an overview of the current knowledge as well as our own work regarding the various aspects of pathogens in the VBNC state.

THE PREVALENCE OF *ESCHERICHIA COLI* PRODUCING B-LACTAMASES IN RETAIL POULTRY MEAT IN LITHUANIA

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Despite the fact that the fifth generation of cephalosporins has already been developed, bacteria are able to discover new mechanisms of resistance. *Escherichia coli* resistant to the second or third generation cephalosporins becomes a particularly serious issue in human medicine, since a wide spectrum of these antibiotics has been developed and used. Such resistant *E. coli* produce β -lactamases, which significantly reduces the spectrum of antibiotic choices. Moreover, resistant zoonotic bacteria circulate among humans and many animal species.

The aim of this work was to investigate the prevalence of *E. coli* producing β -lactamases in retail chains selling fresh raw poultry products in Lithuania. Investigations were performed in 2018 and 2020 at the National Food and Veterinary Risk Assessment Institute. Broilers were obtained at retail markets and tested for the presence of *E. coli* phenotypes producing extended spectrum β -lactamases (ESBL) and *AmpC* type cephalosporinases.

From 300 samples tested, 177 samples (59%) were positive for the presence of *E. coli* producing β -lactamases. From those isolates, 56% belonged to ESBL and 43.5% to the *AmpC* phenotype. All β -lactamases producing isolates were resistant to aminopenicillins and cefotaxime, but all of them were susceptible to carbapenems, temocillin and tigecycline. Frequent resistance was detected towards ceftazidime (98.5%), cefepime (89.5%), fluoroquinolones (75.5%), sulphonamides (75.0%) and tetracycline (69.0%). One isolate was resistant to colistin.

In conclusion, raw meat of broilers is frequently contaminated by *E. coli* resistant to β -lactams and some other classes of medically important antibiotics, including critically important ones.

Keywords: one health, antimicrobials, antimicrobial resistance, broilers, esbl, β -lactamases.

IMPACT OF RAINFALL EVENTS ON COMPLIANCE OF BIVALVE MOLLUSC, HARVESTED IN SARDINIA, ITALY, FOR *ESCHERICHIA COLI*

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Rainfall is one of the most important factors of increased levels of *Escherichia coli* in bivalve molluscs at production stages. Public health control strategies applied by competent authorities rely on microbiological risk assessment, which is based on the evaluation of the sources of faecal contamination in proximity to bivalve molluscs harvesting areas. The monitoring of indicator microorganism, such as *E. coli*, for the presence of bacterial and viral pathogens is essential for the determination of the sanitary status of production areas. The present study was aimed to investigate the impact of rainfall events, in terms of abundance and time before harvesting on the content of *E. coli* in bivalve molluscs farmed in Sardinia (Italy). Enumeration of *E. coli* was performed according to the most probable number (MPN) method (ISO 16649-3) on 1920 bivalve samples collected from 7 regional counties between 2018 and 2020. Precipitation history data were obtained from the local meteorological agency (ARPAS). The cumulative rain (mm) in the 7 days before sampling showed the highest correlation with *E. coli* content in bivalve molluscs. The likelihood of non-compliant samples for *E. coli* increased with the increase of total mm of rain poured in the 7 days before sampling. When the total rain was > 300 mm, 80.5% of the samples were not compliant for *E. coli* with an odds ratio of 23.6. Precipitation data could be a useful tool to interpret anomalous results from official control authorities and to plan harvesting after a rainfall event on a science-based approach.

Keywords: one health, microbiological monitoring, sanitary status, shellfish.

ANTIBIOTIC RESISTANCE OF STAPHYLOCOCCI ISOLATED FROM BULK TANK MILK SAMPLES

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The prevalence of staphylococci in the environment of humans, including food, may lead to the production of toxins and food poisoning in consumers. Additionally, staphylococci constitute a reservoir of genes determining antibiotic resistance. The study aimed to compare the antibiotic resistance of staphylococci isolated from bulk tank milk (milk from healthy cows, meeting the requirements for milk intended for food purposes) and assess the occurrence of animal-associated methicillin-resistant *Staphylococcus aureus*. The study consisted of isolation of strains from cow milk, confirming their belonging to *Staphylococcus* sp. and *S. aureus*, investigation of the presence of a gene determining the production of coagulase, and genes determining resistance to β -lactams, tetracyclines, vancomycin, natural penicillins and aminopenicillins. There were 56 bacterial strains isolated, which belong to *Staphylococcus* spp. All isolates showed resistance to amoxicillin, erythromycin and tetracycline. The lowest resistance was demonstrated for vancomycin (48.2%). The *mecA* gene was not detected in any of the isolates, and there was no positive reaction to the PB2 protein, which indicates that none of the isolated strains was methicillin resistant. Special attention should be paid to the health of dairy cows and supervision of the use of antibiotics in the dairy herd environment because even in milk from healthy cows there is a real risk of the occurrence of multi-drug resistant bacteria.

Keywords: antibiotics, resistance, bulk tank milk, *Staphylococcus* sp., *Staphylococcus aureus*.

CHARACTERISTICS OF *YERSINIA ENTEROCOLITICA* ISOLATED FROM FRESH LETTUCE AND VEGETABLES IN LATVIA

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Yersinia enterocolitica is a foodborne pathogen which may cause yersiniosis – one of the most topical foodborne zoonosis in the European Union. Pork and fresh produce have been implicated in foodborne outbreaks of yersiniosis, and *Y. enterocolitica* has been isolated from retail pork and lettuce. The aim of the present study was to detect the genetic diversity and virulence potential of *Y. enterocolitica* isolated from lettuce and vegetables at retail outlets in Latvia. Altogether, 66 *Yersinia* isolates from fresh lettuce and vegetable samples were investigated by whole genome sequencing. All studied *Y. enterocolitica* strains belonged to non-pathogenic biotype 1A according to the molecular biotyping results. Among identified virulence genes, *inv* (95.4%) and *ystB* (98.5%) were classical virulence genes associated with biotype 1A. However, presence of the chromosome-encoded *ail* virulence gene was also identified in 4.6% of isolates. Core genome multilocus sequence typing revealed a high degree of genetic diversity among *Y. enterocolitica* isolates, and the isolates were not genetically related to each other. Genomic comparisons with recent isolates of animal origin did not reveal a connection between animal and plant-based food isolates. Although the majority of isolated *Y. enterocolitica* strains were lacking classical chromosomal and plasmid-encoded virulence determinants, the presence of *ail* gene may indicate potential virulence of some *Y. enterocolitica* isolates isolated from vegetables.

Keywords: WGS, virulence genes, vegetable, lettuce, *Y. enterocolitica*.

ESBL-PRODUCING *ESCHERICHIA COLI* IN A BROILER FARM: VACCINATION EFFECT ON BACTERIA OCCURRENCE AND ANTIMICROBIAL RESISTANCE

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Escherichia coli, an inhabitant of the avian gastrointestinal microbiota, has emerged as a carrier of extended-spectrum β -lactamase (ESBL) genes, conferring resistance to a multitude of antibiotics. ESBL *E. coli* raises substantial worries for animal health and the risk of transmitting resistant strains to humans through the food chain. Vaccination of broiler flocks against *E. coli* presents a promising intervention to reduce the occurrence of antibiotic-resistant strains.

In total, 400 cloacal and 60 farm environmental swab samples from vaccinated with Poulvac[®] live vaccine and non-vaccinated broiler flocks in a Lithuanian poultry farm were tested for ESBL *E. coli* occurrence. Presumptive ESBL *E. coli* was confirmed, and antimicrobial resistance was evaluated using the combined antibiotic disc and disc diffusion test methods following EUCAST (2017) guidelines.

ESBL *E. coli* was detected in 29.5% of cloacal swab samples from vaccinated broilers compared with 33.5% in non-vaccinated broilers ($P < 0.05$). Altogether, 8.33% of environmental swab samples were positive for ESBL *E. coli* in the vaccinated flock, compared with 13.33% in the non-vaccinated flock. In total, 246 ESBL *E. coli* isolates were tested for resistance to 12 different antibiotics. The study revealed 45 different antimicrobial resistance profiles among tested *E. coli* strains with dominant PX, AUG, CIP, SXT, TE (22.4%) and PX, CRO, AUG, CIP SXT, TE (10.6%) resistance profiles. Further research is needed to fully understand the Poulvac[®] vaccine effect on ESBL *E. coli* occurrence and spread of antimicrobial resistance at farm level.

Keywords: ESBL *E. coli*, antimicrobial resistance, vaccination, poultry.

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