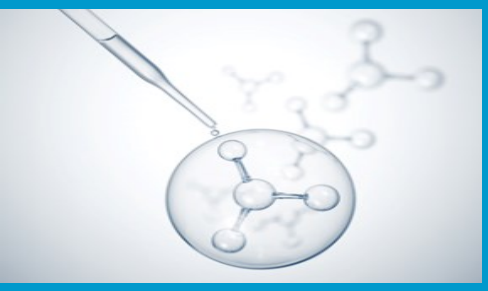
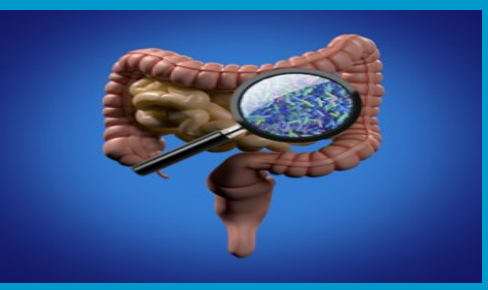
September 22-24 2022, The Royal Hotel, Hammamet, Tunisia

Book of abstracts





THIRD INTERNATIONAL SYMPOSIUM ON NATURAL ANTIMICROBIALS:

Current status, challenges and perspectives

ANTIMIC 2022

3rd INTERNATIONAL SYMPOSIUM ON NATURAL ANTIMICROBIALS: CURRENT STATUS, CHALLENGES AND FUTURE PERSPECTIVES

September 22-24 | Hammamet, Tunisia

PROGRAM

THURSDAY, SEPTEMBER 22, 2022

9h00	Welcome
9h15	**Jean-Yves Madec**, ANSES, Lyon, France *Antimicrobial resistance and environmentally related issues*
10h00	**Flash Presentations** • Ameni Ben Salem*: Antibiotic resistance of Escherichia coli strains isolated from broiler chickens - Emergence of ESBL/AmpC strains in farms of the governorate of Sfax* • Sana Dhaouadi: *Chemical composition and in vitro antimicrobial activity of Cupressus sempervirens and Eucalyptus camaldulensis essential oils against colistin-resistant Escherichia coli and methicillin-resistant Staphylococci recovered from different animal diseases in Tunisia* • Samia Azabou: *Assessment of the anti-biofilm activity of traditional homemade prickly pear vinegar against selected food-borne pathogens on different abiotic surfaces*
10h30	**Coffee break and poster session**
Session 1 : Antibiotic resistance and alternatives in a one world, one health approach **Moderators : Lilia Messadi, ENMV, Tunisia and Karim Ben Slama, ISSBAT, Tunisia**	
11h00	**Carmen Torres**, University of La Rioja, Logroño, Spain *Antimicrobial resistance from the One Health perspective. The case of methicillin-resistant Staphylococcus aureus.*
11h30	**Ilhem Boutiba**, Université de Tunis El Manar, Tunis, Tunisia *Antibiotic resistance in Tunisia: surveillance, status and means of prevention*
11h50	**Ramzi Boubaker Elandoulsi,** University of Manouba, Ariana, Tunisia *Methicillin-resistant Coagulase-Negative Staphylococci: key role in the maintenance and dissemination of antibiotic resistance in human, animal and environment interface*
12h00	**Samia Bouhamdi,** Institut de la recherche vétérinaire de Tunisie, Tunis, Tunisia *Effect of a diet containing olive leaf powder on broiler chickens infected experimentally with Escherichia coli 078:K80*
12h10	**Wedjène Mansour-Ben Romdhane,** Faculté de médecine IBN EL Jazzar, Sousse, Tunisia *Gram-negative bacilli isolated from humans, animals, foodstuffs, and the environment; molecular proof of spread*
12h30	**Laila Ben Said**, Université Laval, Quebec, Canada *Bacteriocins: An emerging alternative against multidrug-resistant bacteria*
12h50	**Lunch**

THURSDAY, SEPTEMBER 22, 2022 (continued)

14h30	**Houssem Ben Yahia,** Université de Tunis El Manar, Tunis, Tunisia *Detection of linezolid and vancomycin resistant Enterococcus strains isolated from avian cecum in Tunisia*
14h40	**Soufiane Telhig**, Université Laval, Quebec, Canada *The potential of microcins as new therapies. A phenotypical and genomic study of their efficiency regarding Enterobacteriaceae and their resistance mechanisms*
Session 2 : Diversity and structure/activity relationship of natural antimicrobials **Moderators: Sylvie Rebuffat, Muséum National d'Histoire Naturelle, France and Laurent Bazinet, Université Laval, Canada**	
14h50	**Mickael Lafond**, ISM2-Biosciences, Marseille, France *Ruminococcins C: from an exotic structure to clinical properties*
15h20	**Djamel Drider and Rozenn Ravallec**, Université de Lille, Villeneuve d'Ascq, France *Enterocin 14: In vitro, in situ and in vivo activity data*
15h50	**Éric Biron**, Université Laval, Quebec, Canada *Synthesis and pharmacological optimization of bacteriocins and lipopeptides for the development of new antimicrobial agents*
16h10	**Rosa Fernández- Fernández, University of La Rioja,** Logroño, Spain *Competitive studies of four micrococcin P1 producer staphylococcal strains against a methicillin-resistant Staphylococcus aureus strain*
16h20	**Aurore Cournoyer,** Université Laval, Quebec, Canada *Valorization of porcine blood, coproduct of slaughterhouses, through the production of antifungal peptides*
16h30	**Coffee break and poster session**
17h00	**Naïma Nedjar**, Université de Lille, Villeneuve d'Ascq, France *Structure-function relationships of the NKT peptide isolated from slaughterhouse coproducts*
17h20	**Flash Presentations** • Sadika Dkhili: *Bacteriophage therapy: A renewed approach to fight multidrug resistant bacteria* • Mariem Benjemaa: *Improvement of the antimicrobial activity of Thymus capitatus essential oil by nanoencapsulation* • Imen Chatti: *Beta-lactam resistance profiles of Klebsiella pneumoniae clinical strains isolated in the Fattouma Bourguiba University Hospital of Monastir* • Rym Essid: *Preparation, characterization of chitosan nanoparticles loaded with Syzygium aromaticum essential oil and evaluation of its Antileishmanial potential*
17h50	**Poster session**
20h00	**Gala dinner**

FRIDAY, SEPTEMBER 23, 2022

9h00	**Sylvie Rebuffat**, Muséum National d'Histoire Naturelle, Paris, France *Bacteriocins, foreground players in microbial interactions and alternatives to antibiotics*
Session 3 : Natural antimicrobials and their role in modulating microbial ecosystems **Moderators: Ismail Fliss, Université Laval, Canada and Abdellatif Boudabous Chihi, Université Tunis El Manar, Tunisia**	
9h45	**Éric Oswald**, Institut de recherche en santé digestive, Université de Toulouse, Toulouse, France *From the biosynthesis of siderophore-microcins to the fight against the intestinal carriage of multi-resistant enterobacteria*
10h15	**Bernhard Krismer**, University of Tübingen, Tübingen, Germany *Niche competition by human-associated Staphylococcus species*
10h45	**Coffee break and poster session**
11h45	**Séverine Zirah**, Muséum National d'Histoire Naturelle, Paris, France *Stability and effects on the colonic microbiota of microcins, antimicrobial peptides produced by enterobacteria*
12h15	**Ramzi Guerbaa**, Université de Tunis El Manar, Tunisia/Université Laval, Canada *Phenotyping and molecular characterization of bacteriocin-producing Escherichia coli isolated from the gastrointestinal tract of poultry*
12h25	**Abdelmonaem Messaoudi**, Université de Tunis El Manar, Tunis, Tunisia *Inhibitor assessment against the LpxC enzyme of antibiotic-resistant Acinetobacter baumannii using virtual screening, dynamics simulation and in vitro assays*
12h35	**Lunch**
Session 4 : Conception and production of natural antimicrobials **Moderators: Carmen Torres, University of La Rioja, Spain and Rozenn Ravallec, Université de Lille, France**	
13h50	**Jacques Corbeil,** Université Laval, Quebec, Canada *Use of metabolomics and machine learning to counter antibiotic resistance*
14h20	**Laurent Bazinet**, Université Laval, Quebec, Canada *How to induce selective separation of antimicrobial peptides by electrodialysis with filtration membrane?*
14h40	**Salwa Karboune**, Université McGill, Sainte-Anne de Bellevue, Canada *Learning to predict: Exploring fully the compositional diversity of natural ingredients and modulating their structures to improve their antimicrobial/antioxidant properties*

FRIDAY, SEPTEMBER 23, 2022 (continued)

Session 5 : Applications and regulations Moderators: Séverine Zirah, Muséum National d'Histoire Naturelle, France and Éric Biron, Université Laval, Canada	
15h00	**Roundtable on regulatory aspects linked to antimicrobial products**
16h00	**Coffee break and poster session**
16h30	**Paul Ross**, APC Microbiome Ireland, University College Cork, Cork, Ireland *Bacteriocins as alternatives to antibiotics for gut health*
17h00	**Damien Bouchard**, Agence Nationale du Médicament Vétérinaire, Fougères, France *Alternatives to antibiotics in the veterinary therapeutic arsenal: issues and challenges*
17h30	**Ismail Fliss**, Université Laval, Quebec, Canada *Natural antimicrobials as new food additives: regulatory aspects and approval process*
17h50	**Salma Zargouni**, Direction Générale des Services Vétérinaires, Tunisia *Presentation of the National action plan to fight antibiotic resistance*
18h00	**Samira Soltani**, Université Laval, Quebec, Canada *New insights into gastrointestinal stability and toxicity of bacteriocins for potential applications in the food, medical and veterinary sectors*
18h10	**Poster awards and closing remarks**

SATURDAY, SEPTEMBER 24, 2022

An optional guided tour will be offered. The cost of this activity is 50 CAD/35 euros.

Itinerary details

1- Departure from The Royal Hotel at 7h45 AM

2- Tour of Sidi Bou Saïd (duration: 1h30)

3- Tour of the Medina of Tunis (duration: 1h30)

4- End of the guided tour around 12h30*

5- Arrival at The Royal Hotel around 14h00

* A direct transfer to the Tunis Carthage airport at the end of the guided tour can be offered to participants

ANTIMICROBIAL RESISTANCE AND ENVIRONMENTALLY RELATED ISSUES

Jean-Yves Madec[1]

[1]ANSES, Lyon, France

Drivers of AMR include antimicrobial use in humans and animals, and the spread of resistant bacteria and genes within and between these sectors. Besides, pollution by antibiotics and AMR bacteria through industrial, hospital, community and farm waste is also expanding the environment resistome, and even more importantly, has an impact on bacterial population genetics and microbiomes. Therefore, the environment not only plays a role in transmitting AMR already circulating in humans and animals but also in the emergence of new resistance determinants of potential public health relevance. Such evolutionary events remain very hard to predict and trace but we may assume that the selective pressure exerted on the environment by the current practices related to antibiotics at the globe level promotes the mobilization and spread of those genes. On the other side, environmental microorganisms have long been a source of natural antibiotics, highlighting the dual nature of this complex setting. Since the human, animal and environmental habitats are interconnected, and that bacteria often cross species boundaries, no doubt that a One Health approach is needed as a collaborative effort to tackle AMR in people, domestic animals, plants, and all components of the environment. The lecture will review our current understanding of the place of the environment as a whole- including water (rivers, sea, effluents), soils and wildlife- in the global AMR burden and discuss possible actions or strategies that may help mitigate risks of emergence, evolution and spread of AMR, and in the end, reduce its negative impact on Global Health.

ANTIBIOTIC RESISTANCE OF *ESCHERICHIA COLI* STRAINS ISOLATED FROM BROILER CHICKENS- EMERGENCE OF ESBL/AMPC STRAINS IN FARMS OF THE SFAX GOVERNORATE

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Antimicrobial resistance is a worldwide threat to human, animal, and environmental health. This critical situation has been amplified by the intensification of livestock farming, especially for poultry, and antibiotic overuse. Among the resistant bacteria, *Escherichia coli* has become the main host for beta-lactamases. The latter are linked to a phenotype of resistance to diverse antibiotics classes that cause serious therapeutic failures.

The objectives of this study were to (i) determine the prevalence of *E. coli* digestive carriage in broiler chickens raised in the Sfax region, (ii) measure antibiotic resistance rates of the isolates and (iii) identify the major genes involved in β-lactams and colistin resistance.

A total of 139 *E. coli* strains isolated from cloacal swabs of broiler chickens aged 38-46 days and collected between February and May, 2019 from four farms in different regions of the Sfax governorate were studied. The susceptibility of *E. coli* strains to 21 antibiotics was investigated using the agar diffusion method. The presence of genes encoding for beta-lactams, cephalosporinases, and colistin resistance was determined by molecular analysis. Phenotyping results revealed the presence of 127 multidrug-resistant *E. coli* isolates (91.4%) and significant levels of extended spectrum b-lactamase (ESBL) (54%) and AmpC cephalosporinase (22.3%). High resistance rates were observed for tetracycline (99,3%), nalidixic acid (94.2%), cefuroxime (90.6%), amoxicillin (89.9%), enrofloxacin (89.2%), piperacillin (85.6%) and cefalotin (85.6%). Several tested strains (25.9%) were resistant to colistin, a last-resort antibiotic used in human medicine. Moreover, 2.9% of the strains were resistant to ertapenem, an antibiotic not used to treat animals.

Molecular analysis of beta-lactams resistance genes was positive for *blaCTX-M-1* (62), *blaCTX-M-9* (6), *blaSHV* (2), *blaTEM* (2), and the cephalosporinase genes *blaCMY* (26) and *blaAmpC* (5). All C3G-resistant isolates (ESBL and cephalosporinases) tested positive for the *mcr-1* plasmid gene, representing a prevalence of 25.2%.

These alarming results confirm the significant need for rigorous control of antimicrobial use, education of veterinarians about the risk of antibiotic resistance development and of livestock producers regarding the potential hazards of self-medication, an expanded laboratory monitoring of antibiotic resistance, and a follow-up to enable timely implementation of corrective measures.

CHEMICAL COMPOSITION AND IN VITRO ANTIMICROBIAL ACTIVITY OF *CUPRESSUS SEMPERVIRENS* AND *EUCALYPTUS CAMALDULENSIS* ESSENTIAL OILS AGAINST COLISTIN-RESISTANT *ESCHERICHIA COLI* AND METHICILLIN-RESISTANT STAPHYLOCOCCI RECOVERED FROM DIFFERENT ANIMAL DISEASES IN TUNISIA

Sana Dhaouadi[1], Salsabil Chedli[1], Soufiene Chaari[2], Ameur Cherif[1], Ramzi Boubaker elandoulsi[1]

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Context and problematic: Bovine mastitis, calf diarrhea and avian colibacillosis caused by staphylococci and *Escherichia coli*, respectively, are among the most important diseases affecting dairy cows, calves and poultry leading to high economic losses. Emerging resistance to colistin and methicillin in *E. coli* and Staphylococci, respectively, is a growing problem in veterinary medicine. Thus, using natural products such as essential oils (EOs) as an alternative option for the treatment of animal diseases appears to be very promising.

Objectives: This study aimed to determine the chemical composition and to evaluate the antibacterial activity of *Cupressus sempervirens* and *Eucalyptus camaldulensis* Eos against methicillin-resistant Staphylococci and colistin-resistant *E. coli* isolates recovered from cows with mastitis, calves with diarrhea and chickens with colibacillosis.

Methodology: The chemical composition of EOs was analyzed by gas chromatography with mass spectrometry detection. The antibacterial activity of *Cupressus sempervirens* and *Eucalyptus camaldulensis* EOs against *E. coli* and *Staphylococcus* isolates was assessed by disk diffusion test and broth microdilution method. Then, Minimal inhibitory concentration **(**MICs) and Minimal bactericidal concentrations (MBCs) were determined for each of the EOs.

Results: Compounds were identified in *C. sempervirens* and *E. camaldulensis* EOs, respectively: α-Pinène (58,3 and 30,2 %), Cinéole 1.8 (6,25 and 52,1 %), Limonène (2,92 and 5,18%), being predominant. Inhibition zones of EOs in *E. coli* isolates varied from 6 to 18 mm for *C. sempervirens* and 14 to 20 mm for *E. camaldulensis*. In staphylococci, the diameters of inhibition varied from 17 to 35 mm for *C. sempervirens* and 16 to 30 mm for *E. camaldulensis.* MICs for *E. camaldulensis* EO ranged from 80µg/mL to ≥640 µg/mL and from 160 µg/mL to ≥640 µg/mL for *C. sempervirens* EO in *E. coli* isolates. Whereas MICs for *E. camaldulensis* EO ranged from 20µg/mL to ≥640 µg/mL and from 80 µg/mL to ≥640 µg/mL for *C. sempervirens* EO in Staphylococci isolates. MBCs/MICs ratios revealed a bactericidal activity of the two EOs tested.

Conclusion: *C. sempervirens* and *E. camaldulensis* EOs were active against methicillin-resistant Staphylococci and colistin-resistant *E. coli* isolates recovered from diseased animals. These findings showed that the use of these EOs as alternatives to antibiotics might be very promising for the development of new therapeutic options against antibiotic-resistant bacteria in veterinary medicine.

Keywords: Antimicrobial activity, essential oils, *E. coli,* Staphylococci, animal diseases

ASSESSMENT OF THE ANTI-BIOFILM ACTIVITY OF TRADITIONAL HOMEMADE PRICKLY PEAR VINEGAR AGAINST SELECTED FOOD-BORNE PATHOGENS ON DIFFERENT ABIOTIC SURFACES

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Background: One of the most worldwide public concerns facing various food industries is the microbial food-borne disease mainly attributed to biofilms, that account for up to 80% of microbial infections. Biofilms have become a real problem in food industries such as brewing, seafood, dairy, poultry, and meat processing, because they make their inhabitants resistant to antimicrobial agents and cleaning processes. Numerous approaches have been used for biofilm prevention and removal such as, flushing, chlorination, and ultraviolet disinfection. However, these methods lack both effectiveness and safety, and therefore, the development of new and natural anti-biofilm agents seems to be interesting, given that the emergence of antibiotic-resistant bacterial strains as well as the consumer attitude towards the consumption of chemically treated food products have encouraged researchers to find effective alternatives and develop natural antimicrobial agents.

Objectives: To evaluate the antibacterial and anti-biofilm activities of traditional homemade prickly pear vinegar (PPV) against selected food-borne pathogens on different abiotic surfaces.

Methods: Minimum inhibitory (MIC) and bactericidal (MBC) concentrations assays were carried out using the two-fold serial dilution method and MTT assay against Gram-positive (*Staphylococcus aureus* CIP 4.83) and Gram-negative (*Escherichia coli* CIP 54127 and *Salmonella enterica* CIP 8297) bacterial strains. The biofilm inhibitory effect of traditional PPV on initial cell attachment and mature microbial biofilms was investigated on three abiotic surfaces viz., polystyrene microplates, stainless steel, and glass slides.

Results: Obtained results demonstrated that homemade PPV could be an effective antimicrobial agent against both sessile and planktonic cells of *S. aureus*, *E. coli*, and *S. enterica*. PPV was found to be more effective in inhibiting initial cell attachment compared to 24h-preformed biofilms. Besides, it was efficient to reduce the metabolic activity of the tested biofilm-forming bacteria. The anti-biofilm effect of homemade PPV was evaluated on two additional abiotic surfaces such as glass and stainless-steel surfaces, commonly encountered in food processing environments. Results showed that treatment of the studied biofilms with PPV at 2×MIC (% v/v) resulted in a log reduction of the initial biomass. Microscopic analysis on glass surface confirmed PPV inhibitive effect, where a reduction in the dispersed cells was observed.

Conclusion: These findings suggested that homemade PPV could be used as a natural and safe antimicrobial and anti-biofilm agent against food-borne pathogens encountered in the agri-food industries.

ANTIMICROBIAL RESISTANCE FROM THE ONE HEALTH PERSPECTIVE. THE CASE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*

Carmen Torres

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Antimicrobial resistance (AMR) is a global problem which has increased enormously in recent years, compromising the treatment of infections in humans and animals. *Staphylococcus aureus* is a commensal microorganism in humans and many animals, but is also an important opportunistic pathogen, representing methicillin resistant isolates (MRSA), a serious clinical problem. The resistance mechanism involved in most cases is *mecA*-related (encodes PBP2a).

Since 2005 it has been evidenced the emergence of MRSA variants associated with the animal field, and especially with livestock (called LA-MRSA), that are having a great impact on public health. One of the most relevant genetic lineages within LA-MRSA is CC398, mainly related with pigs, although it can also be detected in other farm animals (chickens, turkey, cattle …), and can even be found in free-living animals or in environmental samples. MRSA-CC398 has also been frequently detected in food samples of animal origin (pig, poultry…). The MRSA-CC398 clone can colonize and cause infections in humans, especially in people with professional contact with farm animals (specially pigs) but is also increasingly reported in patients with other types of animal's contacts or without this risk factor. This genetic lineage is especially common in hospitals located in geographical areas with high pig-farming density, and studies related to other farming activities (poultry, cows) are also ongoing. The study of LA-MRSA in the human, animal and environmental environments is an important model to understand the dimension of the One Health approach and will be analyzed through the research on MRSA ST398 carried out by the OneHealth-UR research group. The situation in other countries and continents will also be analyzed.

On the other hand, in 2011 a new mechanism of resistance to methicillin in *S. aureus,* called *mecC*, was described, which encodes the protein PBP2c. MRSA strains with the *mecC* mechanism were initially detected in cattle and people in the livestock environment in the UK. In the following years, MRSA-*mecC* strains were detected sporadically in other food-producing animals and in rare cases of human infections. Nevertheless, different studies, some of them of the OneHealth-UR research group, have shown that MRSA-mecC is mostly detected in the wildlife (red deer, hedgehog, wild rabbits, …), with some sporadic cases in farm animals and humans. Data in this topic of the OneHealth group will be shown together with research of other groups, what will allow to analyze evolutive aspects of the MRSA lineages that are in the human-animal interface.

ANTIBIOTIC RESISTANCE IN TUNISIA: MONITORING, STATUS, AND MEANS OF PREVENTION

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Antibiotic resistance is a global threat to public health that has not spared Tunisia. Indeed, in the last 20 years, our country has been facing an overall increase in bacterial resistance to antibiotics. New resistance mechanisms are emerging and spreading, and many currently available antibiotics are rendered ineffective in most clinical situations. This can be explained by bacterial overexposure to antibiotics linked to their overuse and misuse. Combined with the worldwide deceleration of research in the field of antibiotic discovery, the current situation leads to therapeutic difficulties, especially for vulnerable patients who frequently develop healthcare-associated infections.

Solving this universal problem will necessitate concerted action among the concerned sectors and actors, notably the animal and agricultural fields, the environment, school environment, occupational health, etc. The effort must be global and inter-sectoral.

The Tunisian authorities have called for the development of a national plan of action to preserve the antibiotic efficiency. Accordingly, the One Health concept, implemented through the tripartite collaboration of WHO, OIE, and FAO, and concretized by the WHO global plan, FAO plan of action, and by the OIE antimicrobial resistance fight strategies, summarizes the approach to be implemented to counteract antibiotic resistance. Launched in May of 2015, the WHO plan recommends that all member states develop their inter-sectoral plan to face this challenge, which is now generally recognized as a high priority.

Tunisia complied without delay, creating in 2015 an antibiotic resistance prevention technical committee to develop a national plan of action, which soon became a reality as a result of the collaboration of diverse national and international experts, combined with support from the WHO, FAO, and OIE. This ambitious plan of action was established and validated and approved in September 2020.

Monitoring bacterial antibiotic resistance and improving microbiology laboratory practices on a national scale constitute the major axes of this plan of action. Thus, a national monitoring coordination center and a national reference laboratory were designated, with the primary mission of harmonizing regional data collected according to international standards. Regularly updated national monitoring of bacterial antibiotic resistance data offers the basis for implementing the various action plan activities and their follow-up.

METHICILLIN-RESISTANT COAGULASE-NEGATIVE STAPHYLOCOCCI: KEY ROLE IN THE MAINTENANCE AND DISSEMINATION OF ANTIBIOTIC RESISTANCE IN HUMAN, ANIMAL AND ENVIRONMENT INTERFACE

Ramzi Boubaker Elandoulsi [a], *Sana Dhaouadi* [a,c], *Leila Soufi*[a], *Floriana Campanile* [b], *Ameur Cherif* [a], *Stefania Stefani* [b]

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Antibiotic resistance is an issue that extends beyond human health and can only be tackled with a "one-health" approach, which considers human and veterinary medicine in parallel and in the context of environmental factors. Besides *S. aureus*, other staphylococcal species, so-called coagulase-negative staphylococci (CNS), colonize frequently both animals and humans. They have been described to have rates of resistance to several different antimicrobial classes that surpass those of *S. aureus*, being considered reservoirs of antimicrobial resistance for other more pathogenic bacteria. Moreover, CNS were previously suggested to be themselves the origin of resistance genes that provide resistance to important antimicrobial classes like beta-lactams. There is a gap of knowledge and lack in data concerning antibiotic resistance transmission dynamic. Thus, our research will provide important data concerning the impact of antimicrobial drug misuse in environment, agriculture, and animals. The aims were to i) isolate, identify and genetically characterize antibiotic resistant bacteria from environmental, animal, and human sources; and ii) assess risk factors of transmission of antibiotic resistance genes in order to develop preventive measures and efficient control strategies. After sample collection in different "hot spots" (human, animals, and environment), isolation and identification of resistant bacteria, molecular, genomic, and metagenomic innovative methods have fulfilled. CNS from the three origins carried various resistance genes [mecA, blaZ, tet(K), erm(A), erm(B), msr(A)], suggesting an ongoing genetic exchange among CNS from the three niches. The mecA gene was detected in CNS (n=11) recovered from cows, manure, and humans, whereas the mecC gene (n=3) was only detected in CNS from cows and manure. Various staphylococcal cassette chromosome mec (SCCmec)– SCCmec type I (n=1), II (n=3), IV (n=2), V/VII (n=2) and untypeable (n=3) – and diverse pulsed-field gel electrophoresis (PFGE) patterns were observed in mecA-positive CNS. Otherwise, similar SCCmec types and PFGE patterns were found in methicillin-resistant CNS within different farms and origins, showing the potential of SCCmec interspecies exchange and circulation of the same clones of methicillin-resistant CNS in the human– animal–environment interface. This will allow minimizing resistance genes and/or bacteria transfer from environment to animals and between animals and humans and will contribute for the socioeconomic development of sustainable agriculture as well as animals and human welfare.

Keywords: CNS, Human–animal–environment interface, Clonal dissemination

EFFECT OF A DIET CONTAINING OLIVE LEAF POWDER ON BROILER CHICKENS INFECTED EXPERIMENTALLY WITH *ESCHERICHIA COLI* O78:K80

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(2) Laboratoire de Bio-surveillance de l'Environnement - Faculté des Sciences de Bizerte

The objective of this study was to evaluate the effect of olive leaf powder, used as a food additive, on broiler chickens experimentally infected at one month of age using the *Escherichia coli* O78:K80 strain. Two broiler chicken groups, each containing 10 birds, were infected orally with approximately 1.3 x 10^9 cfu of *E. coli O78:K80.* When the birds of one group (10) were 27 days old, they were fed a diet supplemented with 1 g of powdered olive leaf per kg of food per day until slaughter whereas those of the other group were fed a regular diet. Clinical follow-up post infection showed that birds in the group fed with a diet containing olive leaf powder lost less weight than those in the regular diet group and were colonized more slowly by the pathogen. Microbiological analyses conducted after slaughter demonstrated that the *E. coli* counts in the liver, spleen, and flesh of the birds fed a diet containing olive leaf powder were considerably lower than those from the other group.

Keywords: olive leaf powder, broiler chickens, *Escherichia coli* O78:K80

GRAM-NEGATIVE BACILLI ISOLATED FROM HUMANS, ANIMALS, FOODSTUFFS, AND THE ENVIRONMENT: MOLECULAR PROOF OF SPREAD

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In the One Health context, the antibiotic resistance spread should be analyzed from several perspectives using valid tracers to gain a full understanding of the spread dynamics and develop targeted action plans. In this context, research conducted by our team has described the antibiotic resistance molecular mechanisms in Gram-negative bacilli isolated from humans, animals, foodstuffs, and the environment. Bacteria described as critical by the WHO were studied, and their resistance mechanisms were evaluated. The predominance of enterobacteria and non-fermenting Gram-negative bacilli among the isolates was high; Priority was given to the genes conferring resistance to cephalosporins, carbapenems and colistin. The CTX-M group BLSE is particularly well represented in the isolates from different contexts. Carbapenemases were found in more than one context, encoded by the same plasmids, and in closely related bacteria. We observed resistance to the last-resort antibiotic, colistin, and both plasmid and chromosomal resistances were described. The molecular proof of the spread is clear, and plans of action to fight antibiotic resistance must be based on the monitoring of reliable molecular tracers to enhance the effectiveness of interventions.

BACTERIOCINS: AN EMERGING ALTERNATIVE AGAINST MULTIDRUG-RESISTANT BACTERIA

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In livestock production, antibiotics are used to promote animal growth, control infections, and thereby increase profitability. Even if antibiotics are used judiciously, this does not prevent the emergence of multidrug-resistant bacteria (MDR). Several antibiotics used as growth promoter in animal breeding are identical or closely related to those used in human. Efforts to develop new alternative strategies to control bacterial infections related to MDR are continuously on the rise. Promising alternatives include bacteriocins, ribosomally synthesized peptides by Gram-positive and Gram-negative bacteria, displaying antimicrobial activity against phylogenetically related strains. In this presentation, the inhibitory activity of microcin J25, bacteriocin produced by *E. coli,* nisin, bactofencin and pediocin, bacteriocins produced by Gram-positive bacteria, against multidrug-resistant isolates of *Salmonella*, *Streptococcus* and *Staphylococcus*, will be demonstrated. We will also present an example of the application of a consortium of bacteriocins on the teats of dairy cows in order to reduce the bacterial populations causing bovine mastitis.

DETECTION OF LINEZOLID AND VANCOMYCIN RESISTANT *ENTEROCOCCUS* STRAINS ISOLATED FROM AVIAN CECUM IN TUNISIA

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Background: *Enterococcus* has become a potentially high risk zoonotic opportunistic pathogen that can cause critical public health problems. The ability of these bacteria to acquire antibiotic resistance genes poses a major global threat. The aim of this investigation was to detect and characterize vancomycin and linezolid resistance acquired by enterococci isolated from avian cecum samples in Tunisia.

Materials/Methods: Cæcum chicken samples (n=294) were collected from 49 different Tunisian farms during December 2019 to March 2020. Six caeca per each farm were collected and then mixed in sterile spittoons, constituting a composite sample. More than one colony per sample was taken. A total of 167 isolates were recovered on Slanetz– Bartley agar supplemented or not with vancomycin. All the isolates were identified by MALDI-TOF. Phenotypic antimicrobial susceptibility testing, resistance genotyping and molecular typing by pulsed-field gel electrophoresis (PFGE) were performed.

Results: The identification results showed the predominance *E. faecium* (n=112), followed by *E. faecalis* (n=34), *E. durans* (n=08), *E. hirae* (n=10), *E. gallinarum* (n=2) and *E. avium* (n=1). Linezolid-resistance was detected in five *Enterococcus* isolates. After PCR and sequencing, our results showed that four *E. faecalis* harbored the *optr*A gene and one *E. faecium* harbored the *poxt*A gene. Acquired-vancomycin-resistance was detected in two *E. faecalis* isolates. This resistance was mediated by the *vanA* gene. High rates of resistance to tetracycline, erythromycin and chloramphenicol were also observed. After molecular characterization of the collected *Enterococcus* isolates, our results highlighted that the *tet(M)*, *tet(L)*, *erm(B)*, *msr* and *fexA* genes were detected in most tetracycline, erythromycin, and chloramphenicol resistant enterococci. The molecular typing of linezolid- and vancomycin-resistant isolates, performed by PFGE, showed a high genetic diversity.

Conclusion: This investigation provides insights that avian sector can be a reservoir of vancomycin and linezolid resistant enterococci and could be a potential vector of MDR enterococci transmission. Consequently, the implementation of specific control systems in regional and national surveillance of antibiotic resistant bacteria is becoming mandatory.

THE POTENTIAL OF MICROCINS AS NEW THERAPIES. A PHENOTYPICAL AND GENOMIC STUDY OF THEIR EFFICIENCY REGARDING *ENTEROBACTERIACEAE* AND THEIR RESISTANCE MECHANISMS

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Context: The emergence and spread of multi-resistant bacteria (MDRs) represents a major health risk. A notorious group of bacteria infamous for their capacity of evading and resisting antibiotics are *Enterobacteriaceae*. Microcins, antimicrobial peptides of enterobacteria produced ribosomally and targeting other *Enterobacteriaceae*, are a promising alternative to conventional antibiotics.

Objectives: Study of microcin's potential to inhibit MDR *Enterobacteriaceae* strains, as well as define their spectra of activity; explore possible synergistic/antagonistic effects; elucidate and characterize the mechanisms of resistance, co-resistance, and cross-resistance between microcins and antibiotics.

Materials and Methods: The activity of four microcins: microcin J25 (a transcription-inhibiting lasso peptide), microcin C (a translation-inhibiting nucleotide peptide), microcin B17 (a modified microcin with thiazol oxazol rings) and microcin E492 (a siderophore peptide) was examined against a collection of 54 natural isolates, including several MDRs and covering a variety of serotypes distributed over three species: *E. coli*, *K. pneumoniae* and *S. enterica*. The ability of the four microcins to inhibit the collection of isolates was assessed by MIC and CMB measurements. Their potential synergistic/antagonistic effects were characterized by index FIC measures. The characterization of the resistance phenotypes observed against the tested microcins was conducted by comparison of the gene sequences involved in the mechanisms of action said microcins, as well as a non-targeted study GWAS (Genome Wide Association Study).

Results and discussion: None of the strains tested were able to withstand all microcins at the concentrations tested (≤50 µ g/mL). Microcin C had the broadest inhibition spectrum, while microcin J25 had the most effective antimicrobial activities. Consortia microcins/antibiotics were the most beneficial. MFA analysis of microcin inhibition showed a single correlation between gentamicin resistance and MccJ25 resistance. In parallel, targeted, and non-targeted genomic analyses did not detect a correlation between antibiotic resistance patterns, virulence genes and microcin resistance. Resistance to each microcin was correlated with the presence of mutations in genes involved either in the import of microcin or in metabolism or stress response.

Conclusions: Given the ability of the four microcins to inhibit varied *Enterobacteriaceae* species regardless of their antibiotic resistance gene and virulence genes arsenals. Our data highlights the potential of these microcins as new therapies. In addition, it is possible to overcome the narrow spectra of microcins by combining with each other or other antibiotics, since no antagonistic effects were recorded.

RUMINOCOCCINS C: FROM AN EXOTIC STRUCTURE TO CLINICAL PROPERTIES

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The multi-resistance of pathogens to antibiotics represents an important problem of public-health for human and animal, and the discovery of new antimicrobial molecules to fight bacterial resistant strains is nowadays a world-wide priority. Ribosomally synthesized and Post-translationally modified Peptides (RiPPs) with an antibacterial activity produced by bacteria also called bacteriocins, are part of this new generation of promising antimicrobial peptides (AMP). In this context, exploring the ability of the human gut symbionts to produce bacteriocins playing a barrier effect, we discovered and then produced a new synthetic RiPP from the Gram-positive dominant bacterium *Ruminococcus gnavus*. After characterizing *in silico* the Ruminococcin C biosynthetic genes cluster involved in the biosynthesis, maturation, export and immunity of five RumC isoforms (RumC1 to RumC5), we successfully isolated the five RumC isoforms *in vivo* using a mono-associated rat model, as well as produced the RumC1-5 *in vitro* using the *Escherichia coli* recombinant platform. A deep characterization using molecular and structural approaches and their biosynthetic pathway study, revealed that RumC peptides exhibits four thioether bridges build by a Radical-SAM enzyme. Such a post-translational modification gives to RumC1 an hitherto undescribed 3D structural folding into the sactipeptide family. At a functional level, we have shown that RumC1 is efficient to fight multi-drug resistant-clinical isolates in the micromolar range, and to cure a peritoneal infection in *Clostridium perfringens* challenged mice with a lower dose than the conventional antibiotic vancomycin. Also, while maintaining a global homeostasis of the microbiome, RumC1 exhibits additional beneficial properties for the human host, such as wound healing and anti-inflammatory activities. Our last investigations on the molecular mechanism revealed that, unlike known sactipeptides, RumC1 does not exert a pore-forming mode of action, but penetrates the target bacteria, colocalizes at the septal ring, and exhibits a fast lethal effect with an inhibition of the main macromolecule biosynthetic pathways.

ENTEROCIN 14: *IN VITRO*, *IN SITU* AND *IN VIVO* ACTIVITY DATA

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Enterocin 14 (EntDD14) is a leaderless class IIb bacteriocin produced by *Enterococcus faecalis* 14, a strain isolated from meconium [1]. Purification and characterization of this bacteriocin revealed that it comprises two complementary peptides EntDD14A (MGAIAKLVAKFGWP*IV*KK*YY*KQIMQFIG*E*GW*AINKIIDWIKK*HI) and EntDD14B (MGAIAKLVAKFGWP*FI*KKF*Y*KQIMQFIG*Q*GW*TIDQIEK*W*LKR*H) [2]. Bacteriocin's antimicrobial activity against a panel of Gram-positive and Gram-negative bacteria and microscopic fungi has been studied and the enterocin was active only against a group of genetically related Gram-positive bacteria [1 and new data]. However, this bacteriocin could be of interest for medical purposes due to its activity against potentially dangerous pathogens including *Clostridium perfringens* and *Staphylococcus aureus* [1] and its ability to enhance the activity of antibiotics such as erythromycin, kanamycin [3], and methicillin [4]. To verify these results, we conducted *in situ* studies to establish the EntDD14 efficiency alone or in combination with methicillin in limiting *Staphylococcus aureus* adhesion to human cells Caco-2. EntDD14 has also been shown to reduce the synthesis of interleukins IL-6 and IL-8 [5]. This activity was validated *in vivo* using the murine model "holoxenic NMRI-F". We analyzed the impact of the bacteriocin (EntDD14), alone or in combination with erythromycin, against a methicillin-resistant S*taphylococcus aureus* strain (MRSA) in a murine model infected with this strain. We observed that treatment of the group of mice infected with MRSA (10^8 cfu) and EntDD14 (165 mg/kg), or EntDD14 (165 mg/kg) combined with erythromycin (100 mg/kg) allowed (i) better histopathological protection of the liver, spleen, and colon, (ii) improved body weight recovery, and (iii) a more stable intestinal microbiota in comparison with untreated infected mice or mice treated only with the antibiotic [6].

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SYNTHESIS AND PHARMACOLOGICAL OPTIMIZATION OF BACTERIOCINS AND LIPOPEPTIDES FOR THE DEVELOPMENT OF NEW ANTIMICROBIAL AGENTS

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The increase of antibiotic resistance and its spread raise a major challenge for preventing and treating bacterial infections. Due to this critical public health problem and limited treatment options, the development of innovative antimicrobial agents and new alternatives to antibiotics is crucial, and this has become a high priority worldwide. One promising approach has been searching for antimicrobial agents of bacterial origin, such as bacteriocins and lipopeptides. These peptides show very interesting properties such as high activity, original modes of action, various spectra of action and low cytotoxicity. Despite their huge potential in the agri-food and medical sectors, the use of these peptides remains limited due to difficulties associated with their production and/or stability.

To overcome these limitations, our team uses a combination of synthetic and peptidomimetic approaches to produce peptides, study structure-activity relationships and develop analogues with improved pharmacological properties and stability. As a result of applying this approach to the pediocin PA-1, we have been able to develop an efficient synthesis path suitable for large-scale production and to conduct structure-activity studies in order to develop an analogue with enhance stability. Health Canada approved the latter in May 2020 for use as a technological agent in the food sector. Based on this success, the strategy has been applied to other antimicrobial bacteriocins and lipopeptides. The complete synthesis and the results of structure-activity studies of the bacteriocins pediocin PA-1 and bactofencin A, and of the lipopeptides humimycin and glycinocin will be discussed.

COMPETITIVE STUDIES OF FOUR MICROCOCCIN P1 PRODUCER STAPHYLOCOCCAL STRAINS AGAINST A METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* STRAIN

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Introduction: Bacteria live in communities with complex interactions both including mutualistic and competitive dynamics. Bacteriocins are antimicrobial peptides produced and secreted in natural microbial communities and they have an important role in niche competition[1].

Objective: In this respect, the main objective of this work was to evaluate the potential use of four commensal bacteriocin-producer (BP) staphylococcal strains against a multidrug-resistant (MDR) relevant pathogen, a methicillin-resistant *Staphylococcus aureus* (MRSA, CC398) strain.

Methodology: Four BP staphylococcal strains from different species and origins (one *S. aureus*-water, one S*. hominis*-water, and two *S. sciuri*-meat derived food) were selected from a previous study[2] for competition studies. The bacteriocins produced by these strains were determined by mass-spectrometry and *whole-genome-sequencing* (WGS). Competition studies were carried out with the BP strains (clindamycin-susceptible) against a MDR, MRSA-indicator strain (clindamycin-resistant); non-BP strains (of the same species) were used as negative controls. Fresh culture of competitors adjusted to $1{\cdot}10^8$ CFU/mL were mixed at 1:1 ratio and 10µL was spotted in triplicate on basic medium (BM) agar. Samples were taken at 0h, 24h, 48h, and 72h and serial dilutions were plated on BM with/without clindamycin for the indicator selection. Bacterial ratios of MRSA and the respective competitor BP strains were calculated.

Results and discussion: The presence of micrococcin P1 was identified in the four BP strains by mass-spectrometry and WGS. Nevertheless, differences were observed in the four bacteriocin-gene-clusters, even in the structural gene. The two *S. sciuri* BP strains showed high inhibition against the MRSA-indicator strain at 24h of competition (>99% of BP-grown) and this effect was maintained at 48h and 72h. The BP *S. aureus* strain (methicillin-susceptible/CC130) also inhibited the MRSA-indicator strain at 24, 48 and 72h (BP-growth: 89%, 73%, 100%, respectively), although the kill effect was lower than for *S. sciuri* BP strains. Relevant inhibition effect was only shown after 48h for the *S. hominis* BP strain (52% of BP at 72h). Competition assays carried out with non-BP strains, revealed a prevalence growth of the MRSA-indicator in relation with the BP strains.

Conclusion: Three of the micrococcin P1 producer strains of the *S. sciuri* and *S. aureus* species were able to avoid the growth of the MRSA-indicator strain in competitive studies, being this effect evident at 24h, 48h and 72h of incubation. These preliminary studies indicate the interest of these BP strains as potential modulators to control the growth of MDR bacteria, of great interest in biomedical and food-industry applications.

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VALORIZATION OF PORCINE BLOOD, COPRODUCT OF SLAUGHTERHOUSES, THROUGH THE PRODUCTION OF ANTIFUNGAL PEPTIDES

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Pork processing plants in Québec currently collect approximately 21 million liters of blood annually. The corresponding Canadian volume is approximately 68 million liters[1]. Porcine blood can be easily separated into plasma and cruor, the latter containing blood cells, and therefore, a high concentration of hemoglobin. Cruor hydrolysis by pepsin yields a wide variety of peptides, some of which are biologically active. Studies on the antibacterial activities of hydrolyzed hemoglobin peptides have mostly been conducted on bovine hemoglobin, whereas porcine hemoglobin has attracted very little attention. A peptide called Neokyotorphin (NKT), also found in the porcine sequence, possesses strong antibacterial and antioxidant activities[2]. The goal of the present study was to hydrolyze porcine cruor under conditions that favor NKT production and to characterize the overall peptide population obtained. Moreover, since coloration by heme can limit the potential uses of the hydrolysate *in situ*, decolorizing techniques were applied, and their effects on hydrolysate composition and antibacterial and antifungal activities were compared. The degree of hydrolysis of the hydrolysates was measured, and the peptide population was determined using UPLCMS/MS. Antimicrobial activities were evaluated using agar diffusion tests and Minimal Inhibitory Concentrations (MICs) determination. Important differences were found between the obtained peptide profiles. Heme precipitation resulted in a decrease in the concentration or the disappearance of at least 38 peptides. Antifungal activity against some yeast and mold strains decreased by up to 10-fold post hydrolysates decolorization. No antibacterial activity was detected, despite the presence of active peptide sequences. This study shows that hydrolysis duration and the decolorization step have considerable impacts on the population of obtained peptides. This is also the first demonstration of the antifungal activity of this type of hydrolysate. New peptide sequences were identified and synthesized to confirm and quantify these activities. These hydrolysates will be processed to obtain two enriched fractions: one with antifungal properties and one antibacterial fraction rich in NKT. Our overall aim is to produce new active ingredients from porcine blood that can be used for meat preservation within a circular economy framework.

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VALORIZATION OF AGRI-FOOD PROTEINS BY ENZYMATIC PROCESSES TO PRODUCE BIOACTIVE PEPTIDES AND THEIR APPLICATION: STRUCTURE-FUNCTION RELATIONSHIPS OF THE NKT PEPTIDE ISOLATED FROM SLAUGHTERHOUSE COPRODUCTS

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The agri-food industry is gradually turning its attention towards the utilization of its wastes, which are sources of useful bioactive compounds that could become high-value products. Blood, a slaughterhouse coproduct generated in large volumes worldwide, mainly comprises hemoglobin, a protein containing active peptides released by hydrolysis with porcine pepsin. This hydrolysis was monitored over time to identify and characterize all intermediate and final peptides. This allowed us to understand the hydrolysis mechanisms and peptide mapping of each hemoglobin molecular chain. We then searched for antimicrobial peptides resulting from these hydrolyses using peptide maps. The minimal peptide motif linked to antimicrobial activity was also determined. Peptide structure-function relationships and mechanisms of action were then studied to have improved control over the active peptides production. Since enzymatic hydrolysis is complex, we modeled the enzymatic reactions and developed a mathematical model to predict the ideal conditions for obtaining active peptides. Among these, the α137-141 peptide was found to possess inhibitory activity against a broad spectrum of microbial food contaminants. However, isolation of this peptide from hydrolysates containing more that 100 other peptides appears to be a challenge for its use in the food industry. This can be overcome using electrodialysis with ultrafiltration (EDUF), a selective and eco-friendly approach to separate compounds according to molecular charge and mass. However, in conventional hydrolysis, chemical agents are required to adjust the pH of the solution, and the hydrolysates produced have high salt and mineral contents. To reduce this technical inconvenience, a green technology, the electrodialysis with a bipolar membrane (EDBM), has been proposed, as an alternative method, to produce purified bioactive peptides. The peptide α137-141 thus obtained was found to preserve ground beef, slowing rancidification and the growth of bacteria, molds, and yeast as effectively as the chemical preservative butylated hydroxytoluene (BHT). A study of structure-function relationship of antibacterial peptides to understand their mechanisms of action when they are in contact with a bacterial target also showed the possibility of incorporating them into active packaging.

BACTERIOPHAGE THERAPY: A RENEWED APPROACH TO FIGHT MULTIDRUG RESISTANT BACTERIA

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Context and problematics: *Enterobacteriaceae* are a large family of Gram-negative Bacilli, being *Escherichia coli* one of the most representative species of this family. Some *E. coli* strains have developed mechanisms of pathogenicity; they are considered the most important opportunistic pathogens which mean they can cause human and animal diseases. β-lactams are considered the most powerful antimicrobial agents both in human and veterinary medicine. However, resistance to this class of antibiotics has been reported to increase over time especially in Gram-negative bacteria such as *E. coli*. Antibiotic resistance is considered today a worrying and evolving phenomenon and even a major global public health problem. The importance of the current situation urgently requires another bactericidal alternative of special and collective interest in order to tackle multidrug resistant bacteria.

Objectives: Alternative or complementary treatment is requested. The aim of this study was to explore bacteriophages as potential antimicrobial agents against ESBL-producing *E. coli*.

Methodology: Twenty- four virulent phages were isolated from wastewater samples in Tunisia, purified by the double-layer agar method. The lytic activity of the purified phages was tested using 49 bacterial strains. Phage morphology was visualized by transmission electron microscopy (TEM), and life cycle parameters, physicochemical properties and bacterial challenge assays of phages were carried out.

Results and discussion: From a collection of 24 virulent phages infecting different genera and species of bacteria multiresistant to antibiotics, we selected 3 lytic phages specific to ESBL-producing *E.coli* whose results are: Morphological analyze revealed that the three extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* phages belong to the *Caudovirales* order, *Siphoviridae* and *Myoviridae* family. The isolated phages have broad host ranges that can target two bacterial genus and two bacterial species. These phages were resistant to acid and basic pH. These phages maintained their infectious powers during a storage period of 6 months under different temperature conditions with tolerance to a different temperature range. The use of the phage cocktail SD1, SD2 and SD3 proved to be promising in the control of *Escherichia coli* infections.

Conclusion: The rapid adsorption, the short latency period, the enormous quantity of phages released from the infected host, the low frequency of Bacteriophage Insensitive Mutants (BIMs) and the broad host ranges with a wide tolerance to temperature; pH and chloroform clearly showed that these three phages were excellent lytic phages with very suitable potential therapeutic applications.

IMPROVEMENT OF THE ANTIMICROBIAL ACTIVITY OF *THYMUS CAPITATUS* ESSENTIAL OIL BY NANOENCAPSULATION

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Problem and context: Essential oils from aromatic and medicinal plants such as thyme often possess considerable antimicrobial activities. However, these essential oils are unstable, volatile, and easily oxidized, which lead to a loss in their efficiency.

Objective: The aim of this study is to improve the antimicrobial activity of thyme essential oil by stabilizing it in nanoemulsions.

Methods: A response-surface experimental design was used to optimize the process of nanoemulsion of *Thymus capitatus* essential oils. The effect of the encapsulation on the essential oil antimicrobial activity was evaluated using antibacterial activity tests of the crude oil and the optimal nanoemulsion against four bacterial strains (two Gram-negative, two Gram-positive) and a yeast (*Candida albicans*).

Results and discussion: The optimal nanoemulsion had a droplet size of 380 nm, a polydispersity index below 0.5 and was stable during the 15 days storage period. The stabilization process significantly improved the *T. capitatus* crude oil antimicrobial activity. The crude essential oil exhibited a moderate antimicrobial activity against *Salmonella typhimurium* and *Staphylococcus aureus* with growth inhibition percentages between 3 and 17%, respectively. The growth inhibition percentages of all selected strains by the oil encapsulated in a nanoemulsion-based delivery system were significantly higher than that of crude oil. The growth percentages increased from 6 to 53% for *P. aeruginosa,* 17 to 54% for *S. aureus,* 4.6 to 63% for *E. faecalis*, 3 to 52% for S. *typhimurium,* and 6.7 to 69% for the yeast *Candida albicans*.

Conclusion: The antimicrobial activity results revealed that encapsulating the *T. capitatus* essential oil in nanoemulsion delivery system is very advantageous.

BETA-LACTAM RESISTANCE PROFILES OF *KLEBSIELLA PNEUMONIAE* CLINICAL STRAINS ISOLATED IN THE FATTOUMA BOURGUIBA UNIVERSITY HOSPITAL OF MONASTIR

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Introduction: Carbapenemase multidrug-resistant *Klebsiella pneumoniae* infections are an important cause of hospital morbidity and mortality. During this decade, carbapenemase-producing *Klebsiella* have emerged in different regions of the world.

Objective: The objective of this work is to determine, by phenotypic tests, the mechanisms of resistance of *Klebsiella pneumoniae* strains to carbapenems and the nature of the betalactamase responsible for antibiotic resistance.

Materials and methods: Retrospective study covering the period from January 2019 to December 2020. It focused on isolates of carbapenem-resistant *klebsiella pneumoniae* (*Kp*RC) isolated in the microbiology laboratory of the Fattouma Bourguiba University Hospital of Monastir. The bacteriological identification and the antibiogram were carried out by the VITEK technique.

The research of carbapenemases was done by 3 methods such as the Carba -NP test, the CIM test and the combined disc test.

Results: 120 KpRC strains were isolated during the study period, representing a prevalence of 13%. 75% were producers of a class D carbapenemase, 20% of class B and 5% of class A.

Conclusion: Detection of resistance enzymes may help in the process of managing the spread of these multidrug-resistant strains.

Key words: *Klebsiella pneumoniae*, carbapenemases, phenotypic characterization.

PREPARATION, CHARACTERIZATION OF CHITOSAN NANOPARTICLES LOADED WITH *SYZYGIUM AROMATICUM* ESSENTIAL OIL AND EVALUATION OF ITS ANTILEISHMANIAL POTENTIAL

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Background: Essential oils have gained their importance in aromatic, cosmetic and therapeutic field as antimicrobial agents. However, their biological properties are limited due to their volatility, toxic nature, instability, and ease of decomposition under environment or processing conditions. Thus, their encapsulation in matrices is an important tool for their stabilization. The present study aimed to develop, evaluate, and characterize several encapsulating methods of *S. aromaticum* essential oil in order to improve their physicochemical characteristics and enhance their antileishmanial activity.

Materials/Methods: In this work the essential oil of *S. aromaticum* was encapsulated in the nanoparticles of alginate "HE/AL", chitosan "HE/CS" and in the alginate-chitosan complex "HE/AL/CS". The antileishmanial activity of *S. aromaticum* EO nanoparticles was investigated using standard micro-dilution assay and its cytotoxic potential was evaluated against macrophage cell lines RAW 264.7. Formed nanoparticles were characterized by ultraviolet-visible spectrophotometer, Fourier-transform infrared spectroscopy (FTIR) and dynamic light scattering.

Results: *S. aromaticum* EO/AL/CS-NPS exhibited a regular distribution with size range of 849.8 nm. The encapsulation efficiency of this system was more than 83.48%. This nanoencapsulation improved antileishmanial activity (from 15.66 µg/mL to 6.33 µg/mL) and showed a significant decrease of the cytotoxicity against macrophage cells Raw264.7 (four-fold) (SI>1). Moreover, the loading of *S. aromaticum* EO into the complex chitosan/alginate was demonstrated by FTIR spectroscopy by the formation of new bands corresponding to the C-H band at 2922 cm^{-1}. Specific bands of EO on position 1452 and 1402 cm^{-1} were observed in the formed nanoparticles. In addition, the specific band of CS and alginate at 2122 cm^{-1} and 1033 cm^{-1}, respectively was observed in the formed nanoparticles with a shift in the position 2100 cm^{-1} and 1030 cm^{-1}, respectively.

Conclusion: This study revealed that *S. aromaticum* EO nanoencapsulation into chitosan and alginate constitutes a potential delivery system with promising antileishmanial alternative. It stabilizes the physicochemical properties of EO using a very simple ionotropic pre-gelation technique with strong electrostatic interactions and enhances its biological activity.

Keywords: *Syzygium aromaticum* essential oil, nanoencapsulation, chitosan, alginate, antileishmanial activity.

BACTERIOCINS, FOREGROUNG PLAYERS IN MICROBIAL INTERACTIONS AND ALTERNATIVES TO ANTIBIOTICS

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The raise of multidrug-resistant (MDR) pathogens is one of the biggest threats for human health worldwide, and the World Health Organization has warned of a post-antibiotic era, in which common infections will once again kill. Therefore, it is urgent to investigate alternatives to conventional antibiotics, in addition to continuing efforts in discovering novel molecules.

It is currently well established that multicellular organisms live in tight association with complex communities of microorganisms including a large amount of bacteria, which are immersed in interaction networks reflecting the relationships between them and with host organisms. And yet, little is known about the molecules and mechanisms involved. Among them antimicrobial peptides (AMPs), and especially bacterial AMPs called bacteriocins and microcins, have an important role. These ribosomally synthesized peptides, either unmodified or post-translationally modified, display potent activity toward competitors without generating significant resistance under ecological conditions. They have diverse mechanisms of action involving various targets, from the bacterial envelope and the membrane bilayer to most intimate enzymes inside the cell. Beyond their roles in competitions, they act as signalling molecules and contribute in maintaining balanced and dynamic polymicrobial communities (microbiota) in ecological niches. These features make them promising molecules to develop into alternative antibiotic strategies for use in the human and veterinary medicine, agriculture and food industries.

In the context of combating antimicrobial resistance within the One Health framework, where human, animal and environmental healths have to be considered as a whole, the Antimic 2022 conference is timely to bring together the latest advances in understanding the diversity and functions of bacteriocins and other AMPs in complex environments and stimulate multidisciplinary efforts towards their applications. In this presentation, we'll unveil current knowledge on bacteriocins taking select examples, and the principal ecological functions they ensure in different organisms, which are the base for their potent antibacterial properties and their huge potential. We'll also describe current efforts aimed at bringing bacteriocins to applications and address unanswered questions to provide a framework for inspiring future directions of research.

FROM THE BIOSYNTHESIS OF SIDEROPHORE-MICROCINS TO THE FIGHT AGAINST THE INTESTINAL CARRIAGE OF MULTI-RESISTANT ENTEROBACTERIA

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Escherichia coli (*E. coli*) clone ST131 has become dominant over the past 20 years, currently representing 40–80% of extra-pathogenic *E. coli* (ExPEC) strains known to produce extended-spectrum beta-lactamase (ESBL). The intestine is considered their principal reservoir. In most cases, this strain is carried without producing symptoms, which facilitates its spread throughout the population. However, in some situations linked to the hosts (immunosuppression or hospitalization in intensive care) or due to certain bacterial properties, the asymptomatic carriage can degrade to a true infection (meningitis, pyelonephritis, bacteriemia, etc.), especially in residents of long-term care facilities.

We have developed a probiotic-based strategy to limit the intestinal carriage of antibiotic-resistant *Escherichia coli* strains, using *E. coli* strain Nissle 1917 (EcN), which produces siderophore-microcins M and H47. EcN also synthesizes colibactin, a potentially carcinogenic genotoxin. Therefore, we constructed and validated an enhanced version of EcN that is devoid of genotoxic activity but retains its antimicrobial properties and overproduces microcins. We named this strain EcN2.0. We evaluated its efficiency in limiting the digestive carriage of a pathogenic strain of *E. coli* of clone ST131 in a murine model mimicking permanent asymptomatic colonization. The administration of EcN2.0 brought a reduction in individual carriage compared to control animals administrated with a placebo. This study is part of the discovery and development of new alternatives to antibiotics. This therapeutic strategy could be used to limit the intestinal carriage of multidrug-resistant bacteria in both humans and animals.

NICHE COMPETITION BY HUMAN-ASSOCIATED *STAPHYLOCOCCUS* SPECIES

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The microbiomes on human body surfaces affect health in multiple ways since they include not only commensal or mutualistic bacteria but also potentially pathogenic bacteria. Staphylococci belong to the most prominent members of the human microbiome, predominantly colonizing the skin and nares. Besides the most abundant species, *Staphylococcus epidermidis*, the nose is frequently also colonized by the potentially pathogenic *Staphylococcus aureus*. Competition for epithelial attachment sites or limited nutrients, different susceptibilities to host defense molecules and the production of antimicrobial molecules may determine whether different nasal bacteria outcompete each other. We have recently identified lugdunin, produced by nasal *Staphylococcus lugdunensis*, which is able to eradicate *S. aureus*. Interestingly, at the same time this species is dependent on the acquisition of xenosiderophores. But the armory of nasal bacterial isolates includes many more still unidentified compounds, which might play an important role in niche competition. We have now identified epifadin, a highly unstable but broad-acting molecule produced by an NRPS/PKS biosynthetic gene cluster, in various *S. epidermidis* isolates. Constitutive production but short half-live might represent a special strategy in bacterial warfare to reduce unintended collateral damage of supporting co-inhabitants. Unexpectedly, also a well-known molecule from fungi and soil bacteria might play an important role in bacterial rivalry on the human host.

STABILITY AND EFFECTS ON THE COLONIC MICROBIOTA OF MICROCINS, ANTIMICROBIAL PEPTIDES PRODUCED BY ENTEROBACTERIA

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Microcins are bacteriocins produced by enterobacteria. The remarkable diversity of structures and mechanisms of action of several microcins is determined by complex post-translational modifications[1]. They have a relatively narrow spectrum of antibacterial activity and are a promising alternative to antibiotics in view of the emergence and spread of multi-resistance. Microcin J25 (MccJ25), a 21 amino acid peptide produced by *Escherichia coli* has a high antibacterial activity against *Escherichia* and *Salmonella* strains by inhibiting RNA polymerase. Its structure includes an N-terminal macrolactam ring, into which the C-terminal tail bends and is trapped by steric constraints, forming a loop over the ring. We evaluated the stability of MccJ25 and its impact on the colon microbiota *in vitro*, using the TIM-1 dynamic simulator of the digestive tract[2] and the PolyFermS colic fermentation system[3]. Samples were extracted and analyzed using liquid chromatography coupled to mass spectrometry analysis (LC-MS). Multivariate analysis of LC-MS data and generation of molecular networks from LC-MS/MS data were carried out. Obtained results showed that microcin J25 is relatively stable under gastric conditions but is degraded quickly in the compartment mimicking the duodenum. The identification of degradation products formed revealed that the loop underwent multiple hydrolysis. The comparison of the impact of the MCCJ25 and the rifampicin, an antibiotic of the rifamycin family and also an inhibitor of RNA polymerase, on the colonic microbiota showed that the rifampicin had a considerable impact on the metabolome with an accumulation of amino acids linked to inhibition of protein synthesis, whereas metabolic perturbations caused by the action of microcin J25 are short-lived.

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PHENOTYPING AND MOLECULAR CHARACTERIZATION OF BACTERIOCIN-PRODUCING *ESCHERICHIA COLI* ISOLATED FROM THE GASTROINTESTINAL TRACT OF POULTRY

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Context and Problematics: Antibiotic resistance is a major healthcare concern that threatens both animal and human health. The use of antimicrobial peptides is a promising alternative to antibiotics and can contribute to reduce the pressure of selection and thus reducing the spread of drug resistant bacteria.

Objectives: The aim of this work is to characterize *E. coli* strains isolated from avian caecal content of broiler chicken, in terms of antibiotic resistance, virulence profile and capacity to produce bacteriocins. Correlation between virulence and production of bacteriocins will also be studied.

Methodology: A total of 388 lactose positive isolates were recovered from Forty-nine composite samples collected from avian caecal content and identified by biochemical and molecular methods. Upon identification, isolates were screened by direct antagonism against a collection of 10 Beta-lactam resistant enterobacteria. Active isolates were further screened for antimicrobial activity from their supernatant against two Type strains (*E. coli* ATCC 25922 and *Salmonella enterica* Newport ATCC 6269) and two *E. coli* isolates remarkably sensitive to tested supernatants. A subset of strains, selected based on their antimicrobial activity, were phenotypically characterized for susceptibility to antibiotics. Whole genome of these strains was extracted and sequenced.

Results and discussion: Among 388 isolates, 66 were shown to be active by direct antagonism. Screening from the supernatants led to the selection of 21 active strains and 18 non active strains. Antimicrobial susceptibility testing showed an overall high level of resistance to Quinolones (85 %), Tetracyclines (64 %) and Betalactams (94 %). One strain showed resistance to colistin. Genome screening using the resistance genes identifier (RGI) revealed the presence of TEM Betalactamase (26/39) CTX-M Betalactamase (7/39), AmpC Betalactamase (11/39), tetracycline efflux pump tet(A) (1/39), Quinolone-like protein QnrS (3/39), *gyrA* and *parC* mutations (22/39 and 15/39 respectively with co-occurrence in 10/39) , type 3 Amino-acetyltransferase (2/39) and Aminoglycoside nucelotidyltransferase genes *aadA2* et *aadA5* (8/39), sulfonamide resistance genes *sul* (17/39), trimethoprim resistance genes *dfr* (15/39) and colistin resistance gene *mcr-1* (1/39) with an identity percentage ≥95%. No carbapenemase resistance gene were detected. Virulence factors were analysed using the tool VFDB. Relevant APEC (Avian Pathogenic *E. coli*) virulence factors were detected as following: *fimA* (31/39), *iroN* (16/39), *fyuA* (11/39), *irp2* (11/39) and *papC* (5/39). Screening for secondary metabolites gene clusters using ANTISMASH showed presence of biosynthesis gene clusters of the following: Microcin J25 (5/39), Colicin V (15/39), Microcin C7 (2/39), siderophore metabolites (7/39), non-ribosomal peptides (35/39), thiopeptide (29/39) and arylpolyene (5/39).

Conclusion: The obtained results may lead to a better understanding of the involvement of the bacteriocin of *E. coli* in its pathogenicity and persistence in the avian caecal niche.

INHIBITOR ASSESSMENT AGAINST THE LpxC ENZYME OF ANTIBIOTIC-RESISTANT *ACINETOBACTER BAUMANNII* USING VIRTUAL SCREENING, DYNAMICS SIMULATION AND *IN VITRO* ASSAYS

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Acinetobacter baumannii has been ranked in the list of the World Health Organization as the most critical and priority group of the pathogen for which new antibiotics are urgently needed. A total of 3686 proteins retrieved from the *A. baumannii* proteome were subjected to subtractive proteomic analysis to narrow down the spectrum of drug targets. The SWISS-MODEL server was used to perform a 3D homology modelling of the selected target protein. The SAVES server was used to evaluate the overall quality of the model. A dataset of 74500 analogues retrieved from the PubChem database was docked with LpxC using the AutoDock software. In this study we predicted a putative new inhibitor for the Lpxc enzyme of *A. baumannii*. LpxC enzyme was selected as the most appropriate drug target for *A. baumannii*. Results of virtual screening showed that the N-[(2S)-3-amino-1-(hydroxy amino)-1-oxopropan-2-yl]-4-(4-bromophenyl) benzamide (CS250) could be considered as a promising drug candidate targeting the LpxC enzyme. This molecule shows polar interactions with six amino acids and non-polar interactions with eight other residues. In vitro experimental validation was performed through inhibition assay. The results suggest that CS250 could emerge as a promising inhibitory molecule that can be exploited to target this Gram-negative pathogen.

Keywords: Subtractive proteomics, *Acinetobacter baumannii*, virtual screening, LpxC, molecular dynamics simulation

USE OF METABOLOMICS AND MACHINE LEARNING TO COUNTER ANTIBIOTIC RESISTANCE

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Antibiotic resistance is a growing global issue that urgently requires mobilization. Artificial intelligence can be used to identify innovative solutions to the antibiotic resistance. Our objective was to develop rapid and precise tests to evaluate antibiotic resistance, using mass spectrometry coupled with machine learning (MAGITICS project from the JPIAMR). Additionally, we aim to facilitate the development of new antibiotics using artificial intelligence approaches to analyze antimicrobial peptide databases and identify candidates with clinical development potential. We intend to use the generative flow net [(1)] and the robust set covering machine to identify such candidates by modulating the exploration/utilization ratio for new drug discovery.

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HOW TO INDUCE SELECTIVE SEPARATION OF ANTIMICROBIAL PEPTIDES BY ELECTRODIALYSIS WITH FILTRATION MEMBRANE?

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Porcine blood is a major by-product from slaughterhouses. Its precipitated part after centrifugation, cruor, contained an interesting protein called hemoglobin. After enzymatic hydrolysis of hemoglobin, a wide variety of peptides is obtained, especially antimicrobial peptides. To produce fractions concentrated in active peptides, electrodialysis with ultrafiltration membrane (EDUF) was applied. The process already demonstrated the capacity to concentrate peptide, like TSKYR. The present study aimed to evaluate the impacts of different current conditions (pulsed electric field (PEF) and polarity reversal (PR)) on peptide migration selectivity. In the present study, EDUF was used in a cationic configuration, with a 50 kDa molecular weight cut-off UF membrane. Hence, the direct current (DC) condition was compared with combination of pulse/pause duration corresponding to ratios of 1 and 10 for PEF and PR. The peptide populations in the recovery compartments were analyzed using UPLC-MS/MS and peptide relative abundances were compared between conditions. A principal component analysis (PCA) and a hierarchical cluster heatmap were performed to determine potential groups of peptides and to evaluate the impact of current conditions on peptide population. It occurred that PR ratio 1 generated the most significant differences concerning the peptides population migrated while PEF ratio 1 was the second most different. PEF and PR ratio 10, and DC had similar migrated peptide populations. A linear discriminant analysis (LDA) based on the different groups formed and 6 physicochemical characteristics of the peptides allowed to conclude that the main differences were explained by the charge at pH 9, the molecular mass, and the mass/charge ratio. Indeed, DC, PEF and PR at ratio 10 allow the migration of mainly cationic peptides as expected, while PR ratio 1 allows the migration of some anionic peptides with high molecular masses due to the short polarity reversal. For PEF ratio 1 the peptide population, mainly cationic peptide, was explained in a less important manner by their mass and their mass/charge. Preliminary tests in artificial intelligence via a decision tree approach made it possible to discriminate more precisely the impact of the physical characteristics of the peptides on their migration according to the current conditions. From all these results, it appears that the current condition as well as the pulse/pause combination strongly affect the selectivity of migration during EDUF and consequently may impact the final bioactivity. The next steps of the present study will be to evaluate the antimicrobial activities of the fractions produced in the different conditions of current.

LEARNING TO PREDICT: EXPLORING FULLY THE COMPOSITIONAL DIVERSITY OF NATURAL INGREDIENTS AND MODULATING THEIR STRUCTURES TO IMPROVE THEIR ANTIMICROBIAL/ANTIOXIDANT PROPERTIES

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Because of growing consumers' demand for natural, safe and preservative-free products, plant-derived ingredients have gained much attention. In particular, essential oils (EOs) and plant extracts (PEs) are becoming popular natural substitutes for synthetic antimicrobial and antioxidant ingredients as they address consumer concerns regarding the side effects of synthetic ones. The chemical diversity in EOs and PEs composition has been reported, but the comparison across studies, to characterize this diversity and to identify similarity and complementarity between chemical profiles, is difficult, as various techniques and approaches have been used. Understanding the chemical diversity of natural ingredients and their relationships with their antimicrobial and antioxidant properties regardless of their sources are needed in order to maximize their functionalities and optimize their uses as natural ingredients. Herein, in this presentation, I will provide new insights regarding the chemical diversity of EOs and PEs and shed some light on how chemical profiling can be used to identify the inherent synergistic, additive, or antagonistic interactions within or between the natural ingredients through multivariate analyses. Our work evidences that the determination of functional properties based on the chemical profiles can be achieved using predictive models. Our ability to modulate enzymatically the structures of some natural ingredients to limit the effect of their strong flavor profiles or to enhance their solubility will also be discussed.

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BACTERIOCINS AS ALTERNATIVES TO ANTIBIOTICS FOR GUT HEALTH

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There is an increasing need to develop non-antibiotic (and non-chemical) approaches whereby microbiomes (human, animal and food) can be modified in a positive manner from a compositional perspective. At APC Microbiome Ireland, we are adopting a number of strategies to influence the gut and skin microbiome composition and functionality towards improved health outcomes. These include the use of bacteriocins or the strains which produce them (live biotherapeutics) to kill and outcompete undesirable and/or pathogenic organisms. In addition, the ability to produce bacteriocins may offer a competitive advantage to introduced strains thereby improving persistence and/or dominance. We have isolated a battery of strains which produce novel bacteriocins including thuricin produced by *Bacillus thuringensis*, actifensins produced by *Actinomyces* species, homicin produced by coagulase-negative staphylococci and naturally-occurring variants of nisin produced by various organisms. In addition, we have generated a large range of nisin variants with improved properties such as improved activity, bioavailability or activity against Gram negative bacteria. We believe that the development of such pharmabiotics will allow the sculpting of microbiome composition in a precise way to deliver improved functionality for food and health applications.

ALTERNATIVES TO ANTIBIOTICS IN THE VETERINARY THERAPEUTIC ARSENAL: ISSUES AND CHALLENGES

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The current plans of action to fight antibiotic resistance emphasize the significance of developing new therapeutic approaches. Numerous initiatives have explored alternative or complementary treatments to reduce the use of so-called "conventional" antimicrobial agents in livestock production. They include vaccines, antibodies, bacteriophages, peptides, probiotics, and medicinal plant extracts. From a regulatory perspective, many of these substances are considered “frontier” substances that may, depending on the “presentation” or the “function”, fall within legislative jurisdictions encompassing veterinary medicines, biocides, or animal feeds.

Antibiotics have been approved as medicines and can be used for the treatment or prevention of infectious diseases. A substance proposed as a substitute for such treatment should be considered a veterinary drug (therapeutic claim). This category is strictly regulated; development therein requires studies of animal, human, and environmental safety and efficacy studies for the targeted pathology, the development of an industrial-scale manufacturing process, and a quality control system. Commercialization is subject to prior obtaining administrative authorization and permits. Production and wholesale and retail distributions are reserved for suitably staffed business entities that have secured the rights to do so.

Other products may be administered to animals but are subjected to specific regulations.

Biocides: intended to destroy, repel, or render harmful organisms inoffensive, prevent their action or otherwise fight them by chemical or biological means (e.g., disinfectants for veterinary hygiene).

Animal feeds: products formulated to some degree, with or without containing additives. They may be intended for specific nutritional purposes; in which case they are called dietary feeds.

Currently, veterinary medicines regulation is an issue because no specific regulatory framework exists for the evaluation of so-called “alternative” products. The focus of current European thought on this matter aims to develop specific guidelines defining requirements for quality, safety (including the resistance risk) and efficacy to establish the risk/benefit balance of these products. These requirements will have to be sufficiently flexible to encourage the development of new medicines while guaranteeing their safe and efficient use for humans, animals, or the environment, and thereby achieving the overall goal of reducing exposure to antimicrobial agents.

NATURAL ANTIMICROBIALS AS NEW FOOD ADDITIVES: REGULATORY ASPECTS AND APPROVAL PROCESS

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Bacteriocins are a very heterogeneous group of ribosomally synthesized low molecular weight peptides, exhibiting antimicrobial activity against various microorganisms phylogenetically close to the producing strain. Hundreds of bacteriocins have been isolated and characterized at the physicochemical, biological, and molecular levels. Their inhibitory activity has been widely demonstrated against many microorganisms of interest to the food, medical and veterinary sectors. Paradoxically, very few bacteriocins are currently approved and used on an industrial basis. One of the reasons that would explain this situation relates to the absence of clear regulations governing the approval and use of these active molecules in the different fields of application. As part of this presentation, a review of the different programs and guidelines that can be used for the approval of bacteriocins will be presented. We will also present a concrete example of an application process carried out by our team at Laval University in collaboration with the company Fumoirs Grizzly, which led to the approval by Health Canada of a new food additive containing divergicin M35 as an active bacteriocin, for the long-term biopreservation of fresh and processed fish products.

PRESENTATION OF THE NATIONAL ACTION PLAN TO FIGHT ANTIBIOTIC RESISTANCE

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Resistance to antimicrobial agents has become a global issue, and Tunisia has not been spared. The nation is now committed to fighting this alarming development. A technical committee was created on January 2, 2015, by order of the Minister of Health, to develop a national plan of action in accordance with the "One Health " approach, linking human and animal health, and the environment. With the support and commitment of WHO, FAO, and OIE, the members of this committee have achieved considerable progress towards the four axes of the plan, which are public, medical professionals, and legislators' sensitization to the dangers of microbial antibiotic resistance, monitoring of resistance to antimicrobial agents, prevention and control of infections, and judicious use of antimicrobial agents in human and veterinary medicine.

The first steps of the plan of action include the implementation of the four main strategic objectives. Much has been achieved to date, albeit on a fragmented basis. Beyond these specificities, the plan to fight antibiotic resistance spread in the veterinary field requires a comprehensive approach and an intersectoral coordination.

NEW INSIGHTS INTO GASTROINTESTINAL STABILITY AND TOXICITY OF BACTERIOCINS FOR POTENTIAL APPLICATIONS IN THE FOOD, MEDICAL, AND VETERINARY SECTORS

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Introduction: Bacteriocins have received substantial attention for potential applications in the food, veterinary and clinical settings. In order to be used for different applications, bacteriocins have to pass through strict toxicity evaluation to be legally approved. Although bacteriocins have been widely studied for their inhibitory activities, there is not sufficient data available on their gastrointestinal behaviour and toxicity for their intended use [1].

Objective: In this study, different *in vitro* models were used to provide scientific data and a complete portrait of gastrointestinal stability and oral toxicity as well as dermal toxicity (cytotoxicity, skin sensitization, and skin irritability) of selected bacteriocins, namely, microcin J25, pediocin PA-1, nisin Z, bactofencin A.

Method: All bacteriocins have been produced at a high level of purity. Their activity has been monitored using both agar diffusion and microtitration assays. Gastrointestinal stability of selected bacteriocins has been evaluated using *in vitro* simulated digestion according to INFOGEST protocol[2]. Also, hemolytic activity on rat erythrocyte was assessed and by LDH release assay, bacteriocins' interaction with epithelial cells (Caco-2 cells) was studied. Dermal cytotoxicity was assessed using the neutral red and LDH release assays on normal human epidermal keratinocytes (NHEK) cells. Skin sensitization and skin irritability were studied according to OECD guideline using human cell line activation test (h-CLAT assay)[3] and EpiDerm™ culture (EPI-200)[4] as a human skin equivalent, respectively.

Results: Pediocin PA-1, bactofencin A, and nisin were observed to lose their stability passing through the gastrointestinal tract, while microcin J25 is only partially degraded. Besides, selected bacteriocins were not toxic to Caco-2 cells, and the integrity of the cell membrane was observed to remain unaffected in the presence of these bacteriocins at concentrations up to 400 µg/mL. In the hemolysis study, pediocin PA-1, bactofencin A, and nisin were observed to lyse rat erythrocytes at concentrations higher than 50 µg/mL, while microcin J25 showed no effect on these cells. NHEK cells' viability and membrane integrity remained unaltered after exposure to bacteriocins at concentrations up to 400 µg/mL. Furthermore, microcin J25 showed no skin sensitization at concentrations up to 100 µg/mL, while pediocin PA-1, bactofencin A, and nisin Z caused sensitization at concentrations higher than 100 µg/mL. Tissue viability was unaffected in the presence of bacteriocins at concentrations up to 200 µg/mL.

Conclusion: The current study provides scientific evidence that pediocin PA-1, bactofencin A, and microcin J25, could be safely used in the food, medical and veterinary applications orally and topically.

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PREVALENCE AND MECHANISM OF ANTIBIOTIC RESISTANCE IN STAPHYLOCOCCI STRAINS FROM CAECAL OF CHICKENS IN TUNISIA

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Background: The misuse of antibiotics in animal husbandry has been associated with the development and spread of antibiotic-resistant organisms including commensals.

Objective: To determine the prevalence, antimicrobial resistance genotype, and virulence content of *Staphylococcus* spp isolated from traditional and industrial chickens in Tunisia.

Methodology: One hundred and fifty-five individual caecal samples (106 traditional and 49 industrial chickens) were collected from different regions of Tunisia and processed for staphylococci recovery. The identification was determined by the MALDI-TOF-MS platform. The antimicrobial susceptibility phenotype and genotype were investigated by disk diffusion method and by PCR, respectively. The virulence genes *luk-F/S-PV*, *eta*, *etb* and *tst* were investigated by PCR.

Results: The prevalence of caecal staphylococcal carriage was 35.4% and 64.6% among the traditional and industrial chickens, respectively. A total of 48 isolates were identified as staphylococci. Most of these (97.9%) were coagulase-negative staphylococci (CoNS); *S. condimenti* (n=20), *S. arlettae* (n=1), *S. hominis* (n=1), *S.* haemolyticus (n=1), *S. lentus* (n=1), *S. piscifermentans* (n=1), *S. simulans* (n=2), *S. sciuri* (n=10), *S. succinus* (n=3), *S. pasteuri* (n=4) and *S. epidermidis* (n=3). The single *Staphylococcus aureus* identified in this study was methicillin susceptible (MSSA) and belonged to the genetic lineage t6955/CC30. Half of the staphylococci isolates showed a multidrug resistance phenotype. The isolates showed resistance to the following antimicrobials (percentage of resistance/antimicrobial resistance genes detected): penicillin (47.9 %/ *blaZ*), erythromycin and/ or clindamycin (62.5%/ *ermA, ermC* and *msrA*), tetracycline (14.6%/ *tetK, tetL* and *tetM*), and chloramphenicol (18.6%/ *fexA*). Interestingly, the MSSA isolate was *tst*-positive.

Conclusion: High prevalence of fecal carriage by multidrug-resistant staphylococci was found among traditional and industrial chickens in Tunisia. The detection of *tst*-carrying MSSA suggests the need for further surveillance of chicken meat before human consumption.

PREVALENCE AND MOLECULAR CHARACTERIZATION OF EXTENDED-SPECTRUM–β LACTAMASE AND CARBAPENEMASE-PRODUCING *ENTEROBACTERALES* FROM TUNISIAN SEAFOOD

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Multi-resistance to antibiotics in gram-negative bacilli and particularly in *enterobacteriaceae* has become frequent in hospitals in Tunisia. However, data on antibiotic resistant bacteria in aquatic products are scarce. The aims of this study are to estimate the proportion of ESBL- and carbapenemase-producing Enterobacterales in seafood (clams and fish) in Tunisia, and to molecularly characterize the collected isolates. Two types of seafood were sampled in unrelated markets in four different regions in Tunisia (641 pieces of farmed fish and 1075 Mediterranean clams divided into 215 pools and each pool contained 5 pieces). Once purchased, all samples were incubated in tubes containing peptone salt broth for 24 to 48h at 37°C. After incubation, overnight cultures were isolated on selective MacConkey agar plates supplemented with either imipenem or cefotaxime, identified using API20E test strips (bioMérieux, Marcy-l'Étoile, France) and confirmed by Maldi-TOF MS. Antimicrobial susceptibility was determined by the disk diffusion method on Mueller-Hinton agar plates and results were interpreted according to CA-SFM 2021. ESBL-producing Enterobacterales were detected using the Double Disc Synergy Test (DDST). Carbapenem-resistance was detected using an ertapenem disk and was respectively confirmed using the ROSCO KPC/MBL and OXA-48 Confirm Kit (ROSCO Diagnostica, Taastrup, Denmark). DNA was extracted using a NucleoSpin Microbial DNA extraction kit (Macherey-Nagel, Hoerdt, France), according to the manufacturer's instructions. Resistance genes were determined using the CGE online tools. The replicon content and plasmid formula were identified from the WGS data using PlasmidFinder 2.0.1 and pMLST 2.0. From farmed fishes, nine ESBL-producing strains (9/641, 1.4%) were isolated, which were identified as *E. coli* (n=6) and *K. pneumoniae* (n=3). Among the 215 pools of 5 clams analyzed, 18 ESBL-producing isolates were identified, including 14 *E. coli* and 4 *K. pneumoniae*. A low isolation rate of ESBL-producing Enterobacterales was detected 1.6% (18/1075) in clam pools. In fish, the ESBL phenotype was due to the presence of the $bla_{\text{CTX-M-15}}$ gene in all nine isolates but no carbapenemase gene was identified. In clams, the predominant ESBL phenotype was $bla_{\text{CTX-M-1}}$ (n=6/18). blaCPE (NDM1, OXA48) was detected only in 3 *K. pneumoniae's* isolates. Replicon typing on the strains carrying the ESBL and carbapenemase gene revealed that the major type plasmid carried ESBL were IncF (42.3%) [n=11/26]. In all, our results suggest that seafood can be a reservoir of multi-drug resistant bacteria, most probably of human origin but also by selection pressure of antibiotic. Our findings raise concerns that seafood bought for consumption may serve as potential reservoirs of AMR genes and pose serious threat to public health.

ASSESSMENT OF ANTIMICROBIAL POTENCIES OF TRADITIONAL TUNISIAN VINEGARS AGAINST PATHOGENIC MICROBES AND APPLICATION IN MEAT PRESERVATION

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Microbial contamination and growth play important roles in spoilage and quality loss of meat products. Therefore, the aim of the present study is the production of traditional vinegars from four Tunisian fruits, namely grape (GV), fig (FV), prickly pear (PPV) and date (DV) vinegars and the evaluation of their antimicrobial activity against Gram-positive and Gram-negative bacterial strains, as well as against some fungal strains. Their effects on meat preservation have been also investigated. The antibacterial activity of vinegar samples was tested against three Gram-positive (*Staphylococcus aureus* (ATCC25923), *Enterococcus faecalis* (ATCC25912), and *Listeria monocytogenes* (ATCC15313)) and one Gram-negative (*Escherichia coli* (ATCC25922)) bacterial strains using the well diffusion assay. The antifungal activity was evaluated against six fungal pathogens including *Fusarium equiseti* (MK397285), *Trichothecium roseum* (MK397318), *Nothophoma quercina* (MK397282), *Penicilliusum atraense* (MK397314), *Paecilomyces variotii* (MK397308) and *Alternaria alternate* (MK397289). Regarding the effect of vinegars on meat preservation, turkey meat pieces were inoculated with the studied foodborne pathogenic strains and samples were enumerated after inoculation and after margination with homemade vinegars. The obtained results revealed that vinegar samples exhibited significant antimicrobial activity against *E. coli*, *S. aureus*, *E. faecalis* and *L. monocytogenes*. DV showed the most effective inhibition of *S. aureus* with a zone diameter of 24 ± 1.42 mm while GV showed the smallest inhibition zone (10 ± 1.42 mm). Added to that, GV was able only to inhibit the growth of *S. aureus* unlike the other vinegar samples which inhibit the growth of all the tested pathogenic bacteria. For antifungal activity, results showed that only FV and PPV were able to inhibit the growth of only *Fusarium equiseti* and *Trichothecium roseum* strains with zone diameter ranging from 12 ± 0.00 to 14 ± 2.1 mm, and from 11 ± 0.71 to 12 ± 0.5 mm, respectively. Moreover, results indicated that treating meat with vinegar significantly decreased the initial microflora. It can be concluded that vinegar is an effective and safe antimicrobial agent that can be used not only as an antimicrobial agent but also to improve the microbial and sensory quality of meat products.

ISOLATION AND CHARACTERIZATION OF *LACTOBACILLUS REUTERI* FROM AVIAN CECAL CONTENT

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Background: In developing countries, the poultry agro-business sector has largely been floated with indiscriminate use of antimicrobial agents to enhance production. These could consequently encourage the re-emergence and transmission of antimicrobial resistant bacteria to humans.

Objective: This study aims to characterize the antimicrobial resistance profile of *Lactobacillus reuteri* obtained from avian species, isolate, and identify biochemical alternatives that could be useful as antibiotics.

Methodology: One hundred six cecal contents of chicken from different regions of Northern Tunisia were collected, processed (February-September 2021) and *Lactobacillus reuteri* strains were isolated from the Man-Rogosa-Sharpe (MRS) medium. Identification of isolates was carried out by PCR and MALDI-TOF-MS. Antimicrobial susceptibility phenotypes and corresponding genes of all *L. reuteri* isolates were analyzed by disk diffusion and PCR, respectively. The evaluation of the antimicrobial activity was performed by direct antagonistic test on various indicator bacteria.

Results: Seventy-six isolates were identified as *Lactobacillus spp* by PCR. Further analysis by MALDI-TOF-MS revealed that 4 bacteria (12,12 %) out of 33 strains tested show scores of strong similarities to *L. reuteri*. All the *L. reuteri* isolates were susceptible to penicillin and clindamycin. These strains were resistant to erythromycin, gentamycin, sulfamethoxazole-trimethoprim, and ciprofloxacin. Strains X4392, X4393 and X4394 were resistant to tetracycline, vancomycin and tobramycin. For chloramphenicol, these three bacteria exhibit intermediate resistance phenotype. As for the strain X4395, it was susceptible to clindamycin, tobramycin and vancomycin. All the *L. reuteri* strains harbored the *erm(B)* gene, but lacked the *erm(A), erm(C), erm(T)*, *aac(6´)-aph(2”), fex(A)* and *fex(B)* genes. The strain X4395 carried *tet(L)*, *tet(M)* and *tet(K)* genes. However, only *tet*(*L)* was detected in strains X4392 and X4394. Also, all strains have significant antagonistic effects against several indicator bacteria such as *S. aureus* and *Enterococcus spp*.

Conclusion: These data indicate that *L. reuteri* that is supposedly a ‘safe’ probiotic could acquire AMR determinants of human health concerns. It could be inferred that *L. reuteri* presents ‘double-edged sword’ traits, as it presents itself as a reservoir of AMR genes and contains biomolecules that can be useful to fight against some pathogenic bacteria.

CLONAL DISSEMINATION OF METHICILLIN-RESISTANT *MAMMALIICOCCUS SCURI* CARRYING SCC*mecmecC* HYBRID IN FARM ANIMALS FROM TUNISIA

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Context and problematic: *Mammaliicoccus* (formerly *Staphylococcus*) *sciuri* is part of the commensal flora of diverse animals, has been isolated from the environment and has been reported to cause severe infections in animals and humans. However, very little information exists on the molecular epidemiology of *M. sciuri*, as well as the extent of dissemination and the clinical significance of *mecC*-carrying *M. sciuri* isolates.

Objectives: We aimed to assess the extent of dissemination of methicillin-resistant *M. sciuri* in animal farms in Tunisia and evaluate the distribution of virulence and methicillin-resistance genes in the *M. sciuri* population.

Methodology: From May 2016 to June 2017, 152 samples were collected from diseased animals and healthy humans from adjacent farms in Tunisia. Staphylococci and Mammaliicocci were screened by growth in selective media and characterized for antimicrobial susceptibility and biofilm formation, agglutination and hemolysis abilities. Species identification was performed by *tuf* sequencing. Genomic content in antibiotic resistance and virulence genes were analyzed by Whole Genome Sequencing (WGS). *mecC* genomic regions of *M. sciuri mecC* positive strains were aligned and compared with *M. sciuri* (GVGS2) and *S. aureus* LGA251 strains. *M. sciuri* relatedness was inferred using single nucleotide polymorphisms (SNPs) analysis.

Results: *M. sciuri* was the most prevalent species (46.2%) showing the highest resistance rates to fusidic acid (97.3%), oxacillin (73%), penicillin (40.5%), clindamycin (37%), ciprofloxacin (27%) and cefoxitin (24.3%). Some isolates carried genes encoding resistance to as many as nine different antibiotic classes. *mecA* was found in 35% of *M. sciuri* and *mecC* in 16.2%. All isolates carrying *mecC* were of *S. sciuri* subspecies *carnaticus* and carried the hybrid element SCC*mec-mecC*. Several *M. sciuri* were able to produce strong biofilm (27%) and have clumping ability (16%). Additionally, they carried genes for capsule production (*cap8*, 100%), iron-regulated surface determinants (*isdE*, 24%; *isdG*, 3%) and virulence regulation (*clpC* and *clpP*, 100%). SNPs analysis showed that 17 *M. sciuri* cross-transmission events probably occurred between different animal species and farms. Moreover, SCC*mec* was estimated to have been acquired four times by *S. sciuri* subspecies *carnaticus.*

Conclusion: Multidrug-resistant and pathogenic *M. sciuri* were frequently disseminated between different animal species within the farm environment. *mecA* and *mecC* in the farm environment can be disseminated by both frequent acquisition of the SCC*mec* element and clonal dissemination.

DETECTION AND CHARACTERIZATION OF BACTERIOCIN-PRODUCING *ENTEROCOCCUS* STRAINS WITH ANTIMICROBIAL ACTIVITY AGAINST *CLOSTRIDIUM PERFRINGENS*

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Context and problematic: Necrotic enteritis (NE) caused by *Clostridium perfringens* is an emergence issue in poultry farming. New approaches, other than the use of antibiotics, are necessary to prevent the development of multidrug resistant bacteria and NE. Enterococci are commensal microorganisms that might produce antimicrobial peptides (enterocins) with activity against pathogens, being good candidates as probiotics.

Objective: To screen and characterize *Enterococcus* strains from poultry origin for their inhibitory activity against *C. perfringens* and to identify the bacteriocins they produce.

Methodology: 251 enterococci of poultry origin have been used in this study and five bacteriocin-producing enterococci from U. LAVAL collection as controls. First, all strains were screened for their inhibition activity against the indicator *C. perfringens* (X2967) by the "*spot on the lawn*" method. Then, the activity in their supernatants was studied by agar well diffusion and microtitration against a collection of 20 *C. perfringens* strains. Strains showing clear inhibitory activity against one or more indicator strains were further characterized: antibiotic resistance profile, gelatinase and beta-hemolysis activity. Genetic characterization was performed by whole genome sequencing (WGS) to study the genes encoding bacteriocins as well as the resistome, virulome, plasmidome and genetic lineages of bacteriocin-producer isolates.

Results: 12 enterococci showed clear inhibition activity against at least one of the 20 *C. perfringens*. Five of the 12 enterococci were susceptible to the nine antibiotics tested. The remaining strains presented at least resistance to one of the antibiotics being ciprofloxacin 50%, tetracycline 33% and erythromycin 33% the most frequent. The gelatinase activity was positive in one strain and no β-hemolysis was observed. Structural genes for enterocins were detected in the 12 enterococci strains by WGS encoding the following enterocins: Enterocin P, Enterocin L50 A/B, Enterocin A, Enterocin B, Enterocins NKR-5- 3A-D-Z, and Enterocin SE-K4; moreover, genes related to Staphylococcin CSSa/b type were also found. The resistome, virulome and plasmidome of these isolates are being analysed.

Conclusion: With current data, we can conclude that enterococci from poultry origin are enterocin producers and might have potential as probiotics; nevertheless, due to their role as opportunistic pathogens, a deep characterization is needed for determining their potential interest as probiotics.

IN VIVO STUDY OF THE PROMISING POTENTIAL OF BACTERIOCINS AS ALTERNATIVE TO ANTIBIOTICS IN PIG HEALTH

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Context: The use of antibiotics and the development of antibiotic resistance have become a global concern**.** Considering the contribution of animal production to antibiotic resistance, alternatives are currently the focus of much research. Bacterial origin bacteriocins, could be such an alternative. They are ribosomally-synthesized and posttranslationally modified peptides that are naturally produced by the microbiota of many domestic animals as well as that of humans (1, 2). They possess a narrower spectrum of activity than antibiotics, thus reducing both the chance of resistance and collateral damage to the host' microbiota (3, 4).

Objective: The aim of the study is to characterize the effects of the bacteriocins Nisin (NIS) and microcin J25 (MIC) on the health of piglets.

Material and methods: 288 weaned male piglets were blocked by initial weight and distributed into 48 pens of 6 animals. Four dietary treatments (12 pens/treatment) were applied from day 1 through day 21 post-weaning. 1) No antibiotics (NAB; negative control); 2) Antibiotics (AB; positive control; chlortetracycline); 3) Microcin MccJ25 (MIC); and 4) Nisin (NIS). Animal performance was monitored weekly through day 42. Furthermore, feces samples were collected throughout the experiment and analyzed for volatile fatty acids (VFA) and microbiota composition using 16S-RNA gene sequencing.

Results and Discussion: Total fecal VFA concentrations as well as proportions of acetate, propionate and butyrate were not affected by treatment, but increased over time ($P < 0.001$). There was a treatment × time interaction for average daily weight gain (ADWG), which was lowest in NAB relative to the other groups ($P < 0.001$). Interestingly, AB presented the lowest ADWG on week 3, but was not different from other groups at any other time point. Live weight was the lowest for NAB and no weight differences were observed between MIC, NIS and AB groups, except on week 6, where piglets from the MIC group were heavier compared to the NIS ($P < 0.01$). Firmicutes (71 ± 9.9%) and Bacteroidota (27 ± 8.9%) were the predominant phyla in fecal samples, followed by proteobacteria (1.2 ± 3%). Firmicutes were not affected by treatment. Bacteroidota increased over time ($P < 0.01$), and relative to NIS and NAB, Bacteroidota were reduced by AB ($P < 0.05$) and tended to be reduced by MIC ($P = 0.09$).

Conclusions: Comparable growth performance was observed in piglets given different feed antimicrobials suggesting their potential to replace commercial use antibiotics, such as tetracycline.

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ANTIMICROBIAL ACTIVITY OF COMMENSAL *STAPHYLOCOCCUS* SPP. ISOLATES FROM NASAL MICROBIOTA OF STORKS, PETS AND HUMANS

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Context, problematics, and objectives: Bacteriocins are antimicrobial proteinaceous substances secreted by some bacteria, to confer a selective advantage to the producer in terms of niche colonization ability. *Staphylococcus* is a genus widely distributed in the environment that frequently colonize the skin and mucous membranes of humans and animals and has also been described as bacteriocin producer. In this respect, the objective of this study was to analyze the antimicrobial-activity (AA) in a large collection of coagulase-positive and -negative staphylococci (CoPS and CoNS, respectively) isolates of different origins and to characterize the antimicrobial-producer (AP) isolates.

Methodology: AA was evaluated in 272 staphylococci, including 18 different species (CoNS, n=15 and CoPS, n=3) recovered from different origins (storks, n=213; humans, n=33; and dogs, n=26). All the isolates were tested for AA by the *spot-on-lawn* method against 14 indicator bacteria, including multi-drug-resistant bacteria and relevant pathogens. In the AP isolates, cell free supernatants (CFS) sterilized by filtration or boiling and concentrated (by a speed-vacuum or 1-butanol extraction) were obtained. Their AA were analyzed by *agar-diffusion-assay* against the 14 indicator bacteria. For an in deep study of the compounds responsible for the AA, the susceptibility to 5 proteolytic enzymes (trypsin, alpha-quimiotrypsin, protease, proteinase K and papain) of the concentrated extracts was analyzed against a methicillin-resistant *S. pseudintermedius* (MRSP) indicator bacteria.

Results and discussion: Seventeen (13 CoNS and 4 CoPS) isolates from dogs and storks of the 272 staphylococci tested (6.3%) showed AA by the *spot-on-lawn* method against at least one of the 14 indicators tested. Focusing on CFS, only 4 isolates showed AA after filtration and 3 after boiling (inhibiting from 7.1 to 42.8% of the indicator bacteria, in both cases). Moreover, speed-vacuum concentration (n=8 isolates) and 1-butanol extraction (n=13 isolates) revealed AA against the indicator bacteria from 7.1% to 64.3% ranges. Five out of the 17 AP isolates (*S. pseudintermedius*, *S. pasteuri, S. simulans* of dog origin; and *S. hominis* and *S. chromogenes* of stork) were relevant due to their intense AA against relevant indicator bacteria, including methicillin-resistant *S. aureus* and MRSP, among others. In two of them (1 *S. hominis* and 1 *S. pseudintemedius*), enzymatic treatment was performed, and the AA was lost verifying the protein nature of their antimicrobial compounds that will be further characterized.

Conclusion: Nasal staphylococci of storks and dogs, especially CoNS, produce bacteriocins that can be important in the modulations of their nasal microbiome.

ÉVALUATION DE L'ACTIVITÉ ANTIMYCOBACTÉRIENNE DE L'HUILE ESSENTIELLE EXTRAITE DE *LAURUS NOBILIS*

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Contexte et problématique: L'émergence mondiale de la tuberculose et le développement de la résistance aux médicaments du complexe de *Mycobacterium tuberculosis* multirésistants et ultrarésistants, a suscité l'intérêt des scientifiques pour rechercher de nouveaux agents anti-mycobactériens à partir des huiles essentielles de plantes médicinales.

Objectifs : L'objectif principal de notre étude était d'évaluer l'activité antimycobactérienne de l'huile essentielle de *Laurus nobilis* sur les *Mycobacterium tuberculosis* (sauvage et multidrug resistant) et *Mycobacterium bovis* par la technique Resazurin microtiter assay (REMA).

Méthodologie: Pour la détermination de la CMI de l'huile essentielle, nous avons opté pour le protocole suivant : dans chacun des puits de la plaque, nous avons ajouté tout d'abord 100 µl de bouillon Middlebrook 7H9-S, puis 100 µl de l'huile essentielle à CC de 8mg/mL. Des dilutions sérielles doubles d'huile essentielle ont été ensuite préparées directement sur la plaque pour obtenir la plage de concentration de 2 à 0,0019 mg/ml. Finalement, 100 µl de suspension bactérienne diluée au 1 :20 ont été ajoutés dans chaque puit. Après incubation, 30 µl d'une solution fraîchement préparée de résazurine ont été ajoutés à chacun des puits. Un changement de couleur du bleu (état oxydé) au rose (état réduit) indique une croissance bactérienne.

Résultats et discussion: L'huile essentielle utilisée dans cette étude a montré une très bonne activité contre les *Mycobacterium tuberculosis* (sauvage et multidrug resistant) ainsi que les *Mycobacterium bovis* avec une concentration minimale inhibitrice de 100 µg/ml. Un résultat similaire a été trouvé par une autre étude sur *Laurus nobilis* et montrant une inhibition de la croissance des isolats cliniques de *M. tuberculosis* avec différents profils de résistance (CMI de 100µg/ml) [1]. L'activité antituberculeuse de l'huile essentielle de *Laurus nobilis* réside principalement dans la fraction méthanolique. Les composés de cette fraction méthanolique entière à savoir le costunolide et le déshydrocostuslactone sont responsables de l'essentiel de l'activité antimycobactérienne (mais pas exclusivement) avec différents profils de résistance et des CMI allant de 3,25 à 50 µg/ml. Cette activité antimicrobienne dépend non seulement de la composition chimique de l'huile essentielle mais aussi de ses propriétés hydrophobes. [2]

Conclusion: Nos résultats plaident en faveur de l'utilisation d'huiles essentielles extraites de *Laurus nobilis* pour le traitement de la tuberculose mais des études pharmacologiques préalables sont nécessaires pour évaluer son efficacité en laboratoire sur des animaux.

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SYNTHÈSE ET ÉTUDE STRUCTURE-ACTIVITÉ DU LIPOPEPTIDE ANTIMICROBIEN BRÉVIBACILLINE

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Contexte et problématique : L'utilisation abusive des antibiotiques aussi bien chez l'humain que chez les animaux d'élevage a conduit à l'émergence de plusieurs bactéries résistantes aux antibiotiques dont l'impact sanitaire est majeur (1) (2). Face à ce problème de santé publique critique, le développement de nouveaux agents antimicrobiens est devenu une priorité mondiale. Parmi les alternatives prometteuses aux antibiotiques conventionnels, les peptides antimicrobiens (PAMs) produits par les microorganismes sont particulièrement intéressants pour les secteurs agroalimentaire, vétérinaire et médical. La brévibacilline est un lipopeptide produit par *Brevibacillus larterosporus* qui a démontré des activités antimicrobiennes prometteuses contre des bactéries problématiques comme les *Staphylococcus aureus* résistants à la méticilline, *Enterococcus faecalis* résistants à la vancomycine, *Clostridium difficile* et *Listeria monocytogenes*.

Objectifs : Dans le but de développer un antimicrobien efficace contre *L. monocytogenes*, l'objectif était d'optimiser l'activité antimicrobienne et les propriétés pharmacologiques de la brévibacilline en effectuant une étude structure-activité et d'évaluer les effets synergiques possibles avec d'autre antimicrobiens.

Méthodologie : La brévibacilline et ses analogues ont été produits par synthèse chimique sur support solide (3, 4). Des tests de diffusion sur gélose et de microtitration contre *L. ivanovii* et une collection d'isolats alimentaires de *L. monocytogenes* ont été utilisés pour déterminer les concentrations minimales inhibitrices (CMI) et bactéricide (CMB). Le test de l'indice de concentration inhibitrice fractionné a été utilisé pour déterminer les effets synergiques, additifs et antagonistes entre les différentes combinaisons d'antimicrobiens à l'étude.

Résultats et discussion : La brévibacilline et 15 autres analogues ont été synthétisés avec de bons rendements et caractérisés par spectrométrie de masse. L'activité antimicrobienne des peptides a tout d'abord été évaluée contre la souche indicatrice *Listeria ivanovii* HPB28. Les résultats obtenus démontrent que 2 analogues modifiés à la position 1 montrent une activité inhibitrice équivalente à celle de la brévibacilline native (CMI = 1-3 µg/ml). L'étude structure-activité a démontré que certaines positions peuvent être modifiées sans affecter l'activité antimicrobienne. Ces résultats suggèrent que ces positions pourraient être exploitées pour augmenter la stabilité du peptide, accroître les rendements de production et améliorer les propriétés pharmacologiques.

Conclusion : Les résultats obtenus démontrent le potentiel de la brévibacilline pour le contrôle de *Listeria*. L'étude structure-activité démontre qu'il est possible d'améliorer les propriétés de ce lipopeptide qui peut être produit à grande échelle pour une utilisation potentielle dans les secteurs alimentaire, vétérinaire et médical.

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A SURVEY OF POSSIBLE ETIOLOGIC THERMAL WATER'S CONTAMINATION AND TRIALS OF CARRYING NATURAL FLAVORED DISINFECTION

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Context and problematic: Etiologic thermal water's contamination and research of new approach of efficient disinfection.

Objectives: - Early survey of possible contamination of thermal water. -Trials of disinfection using medical and flavouring plants essential extracts and hydrolytes.

Methodology: Water analysis using membrane filtration method is an effective, accepted method for testing fluid samples to inspect microbiological contamination. Such a method allows the isolation and enumeration of microorganisms followed by biochemical characterization. The ability of antimicrobial activity of some Tunisian medicinal plant extracts and hydrolytes is tested by using disc diffusion assay and the minimum inhibitory concentration (MIC).

Results and discussion: Water quality is affected by a wide range of natural and human influences. The most important of the natural influences are geological, hydrological and climatic, since these affect the quality of water available. The result of the survey indicates that the most contaminant found in thermal water is *Enterococcus spp*. and *Pseudomonas*. There is usually a delay between a pollution incident and detection of the contaminant at the point of water abstraction. The plant extracts that had a better inhibition zone were selected and used for MIC tests. Plant extracts exhibited a remarkable activity against bacterial development.

Conclusion: The results obtained in this survey demonstrate that there is a lot of fecal contamination in thermal water which remain a threat for user health. Disinfection efficiency has to be verified. Antimicrobial activities of plant extracts suggest feasibility to be used in disinfection method.

SPREAD OF blaCTX-M-15-PRODUCING ENTEROBACTERIACEAE AND OXA-23-PRODUCING *ACINETOBACTER BAUMANNII* SEQUENCE TYPE 2 IN TUNISIAN SEAFOOD

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Worldwide, the emergence and global spread of Multidrug-resistant (MDR) bacteria is of great concern. The reservoirs for such organisms are increasing, not only in hospitals, but also in the community, environment, in livestock, companion animals and wildlife. A new and important development is the presence of such organisms and aquatic animals (Seafood). Resistance to extended-spectrum cephalosporins, fluoroquinolones and colistin is under constant scrutiny in seafood worldwide. Bivalves are filter-feeding animals and markers of bacterial pollution.

This study assessed the role of bivalves as potential reservoirs of these resistance determinants. The aim of the present study was to characterize extended-spectrum β-lactamase (ESBL), carbapenem and quinolone-resistant Enterobacteriaceae from depurated mussels (*Mytilus galloprovincialis*) and oysters (*Crassostrea gigas*) collected in the Bizerte lagoon, and scallops (*Placopectenmagellanicus*) from the Monastir lagoon. We report a massive spread of blaCTX-M-15 through dominant *Escherichia coli* and *Klebsiella pneumoniae* lineages and/or plasmid subtypes (F31:A4:B1) as well as the presence of OXA-23-producing *Acinetobacter baumannii* sequence type 2 (ST2) in seafood, highlighting a direct risk for the consumer. These findings should urge authorities to consider hospital effluents, and also farm and urban effluents, as important sources of extended-spectrum-beta-lactamase (ESBL)/carbapenemase producers that filter-feeding animals can concentrate and further spread to humans.

Keywords: Seafood, ESBL, carbapenemase, *E. coli*, *Acinetobacter*, OXA-23, CTX-M-15, IncF, plasmid

CHARACTERIZATION AND ANTIMICROBIAL RESISTANCE OF *ESCHERICHIA COLI* ISOLATED FROM HEALTHY CATTLE IN TUNISIA

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Backgrounds: Antibiotic resistance is a major global public health threat. Healthy animals can constitute a reservoir for antibiotic-resistant *Escherichia coli* causing more severe health outcomes in humans. The aim of this study was to assess the antimicrobial resistance among *E. coli* in fecal samples of healthy cattle and to detect their resistance genes and their virulence genes.

Methodology: A total of 236 *E. coli* isolates were recovered from fecal samples of healthy cattle collected from slaughterhouses (n=160) and from five farms (n=100). The antimicrobial resistance was determined by the disk diffusion method using twenty-one antibiotics discs, β-lactamases genes, associated resistance genes, and virulence genes were studied by PCR.

Results: Of the 236 *E. coli* isolates, 12.7% (30/236) were resistant to the 3rd generation cephalosporins-resistant and/or carbapenem. The following resistance genes were identified among the thirty isolates; $bla_{\text{CTX-M-G-1}}$ (50%, 15/30), $bla_{\text{SHV-1}}$ (30%, 9/30), $bla_{\text{TEM-1}}$ (36.7%, 11/30), bla_{IMP} (30%, 9/30), $bla_{\text{OXA-48}}$ (23.3%, 7/30) and bla_{NDM} (3.3%, 1/30). Quinolones resistance genes *qnrB*, *aac(6')-Ib-cr, qnrA qnrs* were found in 10, 6, 5 and 3 isolates, respectively. The virulence gene *fimH* was detected in 17 isolates, the *traT* gene in 12 isolates and the *stx1* gene in 8 isolates. Other virulence factors screened (*cdt3, stx2*, *iutA, sfa/focDE, ehxA* and *hlyA*) were present among the isolates. The studied isolates belonged to phylogroups B1 (21 isolates), D (4 isolates), B2 (2 isolates) and A (2 isolates) and C (1 isolates).

Conclusion: The ESBL and carbapenemase genes were identified from fecal samples of healthy cattle. The findings encourage the need to control the use of antimicrobial agents in veterinary medicine.

Key words: Antimicrobial resistance, *Escherichia coli*; healthy cattle; carbapenemase genes; virulence factors; $bla_{\text{CTX-M-1}}$

ANTIBACTERIAL SUSCEPTIBILITY PATTERNS OF BACTERIAL ISOLATED FROM URINARY TRACT INFECTION OF PREGNANT WOMEN IN SABRATHA TEACHING HOSPITAL

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Urinary tract infections are one of the most common reproductive system infections in women of childbearing age around the world. The current study was conducted using routine bacterial diagnostics and culture to detect *Escherichia coli* bacteria as they may be one of the infection factors causing UTIs in pregnant women, and to investigate the effect of various antibacterial agents on the collected clinical specimens. To achieve the objective of the study, a total of 20 urine samples from pregnant women with fever, vaginal discharge and other clinical symptoms were examined, using different bacterial cultures to identify and diagnose the bacterial isolates. All infected cases were diagnosed by physicians specializing in obstetrics and gynecology or the reproductive system. The clinical isolates of *Escherichia coli* were diagnosed using various bacterial media to identify and diagnose the bacterial isolates. In the study, 19 bacterial isolates were isolated and diagnosed, which were (11) 57.9% *Escherichia coli,* 1 (5.3%) *Staphylococcus*, and 7 (36.8%) *Staphylococcus aureus*. The results of the study showed that only *Escherichia coli* had high sensitivity to ciprofloxacin 79% *in vitro*. On the other hand, while the other antimicrobial agents, amoxicillin, augmentin, and ampin, showed varying degrees of allergy or resistance, the bacteria showed resistance to ampicillin and rosuvin. These results indicate increasing resistance to these antibacterial agents, which are commonly used for urinary tract infections. This leads to an increase in the number, species, and virulence of bacteria, making them resistant to antibiotics and becoming a public health problem. The rate at which bacteria become resistant to antibacterial agents is of public health concern. This requires routine bacteriologic culture and susceptibility testing of urinary tract infections. In addition, adequate health care keeps women free of UTIs and maintains overall human health.

Keywords: Urinary tract infection, pregnant women, *Escherichia coli*

PLAN D'ACTION NATIONAL 2019-2023 : APPUI À LA PREVENTION DE LA RÉSISTANCE AUX ANTIBIOTIQUES

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Les antibiotiques sont des substances capables d'inhiber spécifiquement la croissance de micro-organismes ou de les détruire. Ce sont des substances chimiques produites par des micro-organismes obtenus par semi-synthèse ou synthèse chimiques. L'acquisition de résistance aux antibiotiques est causée par plusieurs mécanismes dont les mécanismes génétiques, mutations ou encore acquisition de gènes de résistance d'où l'apparition de bactéries multi-résistantes contre lesquelles aucun antibiotique n'est efficace.

C'est ainsi qu'en 2015, l'OMS a publié un plan d'action mondial contre la résistance aux antibiotiques, réalisé en coopération étroite avec ses partenaires, l'organisation mondiale de la santé animale (OIE) et l'organisation des nations unies pour l'alimentation et l'agriculture (FAO). L'OMS a recommandé à tous les états membres, d'élaborer des plans d'action intersectoriels, spécifiques à chaque pays. La Tunisie n'a pas été épargnée par ce phénomène. Les autorités nationales ont mis en place un comité technique de lutte contre l'antibio-résistance multidisciplinaire ayant pour mission d'établir une stratégie nationale. Par conséquent un plan d'action national a été établi selon l'approche « One Health » dans laquelle s'intègrent différentes disciplines santé humaine, animale et environnementale.

Mots clés : antibiotiques, antibio-résistance, One Health

ACTIVITÉ ANTIMICROBIENNE D'UNE NANOÉMULSION DE L'HUILE ESSENTIELLE DE *THYMUS CAPITATUS*

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Contexte et problématique : Les activités biologiques, particulièrement l'activité antimicrobienne, des huiles essentielles végétales sont reconnues et approuvées depuis de nombreuses années. Cependant, peu de recherches ont été consacrées à l'étude de l'effet de la stabilisation des huiles essentielles en nanoémulsion sur l'évolution de l'efficacité de leurs activités antimicrobiennes.

Objectifs : Le présent travail vise à suivre la cinétique de l'activité antimicrobienne de l'huile essentielle du thym suite à sa stabilisation dans un système d'administration à base de nanoémulsion.

Méthodologie : Suite à l'encapsulation de l'huile essentielle de *Thymus capitatus* dans une nanoémulsion stable, son activité antibactérienne contre deux souches (*Bacillus subtilis et Escherichia coli*) a été étudiée. Pour ce faire, un suivi de la croissance bactérienne pendant 24h à 37 °C en présence de la naoémulsion a été réalisé pour les deux bactéries. Au cours de cette incubation, un décompte des colonies bactériennes a été effectué à intervalles réguliers. Aussi, deux contrôles négatifs ont été réalisés avec la même méthode, en remplaçant les nanoémulsions par de l'eau physiologique stérile.

Résultats et discussion : Les principaux résultats montrent que les deux nanoémulsions étudiées ont exhibé un effet inhibiteur plus qu'intéressant et ce contre les deux souches bactériennes. D'ailleurs, cette efficacité a été significativement plus prononcée contre la souche *E. coli* que contre la souche *B. subtilis*. Pour ce qui est de *B. subtilis*, l'effet antibactérien a été notable à partir de 12h d'incubation avec une réduction du nombre total de colonies de 435 (pour le témoin négatif) à 118 colonies en présence de la naoémulsion. Cette activité a atteint son maximum d'inhibition après 18h d'incubation où le nombre total de colonies a été limité à 227 contre 524 pour le témoin. Au terme de la période d'incubation, le pourcentage d'inhibition de la croissance de *B. subtilis* par la nanoémulsion du thym est estimée à 57%. Cette tendance a été retrouvée contre *Escherichia coli* avec une efficacité d'inhibition de la croissance plus prononcée. En effet, après 24h d'incubation, l'inhibition de la croissance d'*E. coli* est de 98%.

Conclusion. Les données recueillies au cours de cette étude soulignent le potentiel exceptionnel de l'utilisation des nanoémulsion de l'huile essentielle de *Thymus capitatus* à des fins aussi bien alimentaires que médicales.