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NINTH INTERNATIONAL CONFERENCE ON ANTIMICROBIAL AGENTS IN VETERINARY MEDICINE (AAVM)
Rome, Italy, October 16-19, 2018

International conference

Antimicrobial agents in veterinary medicine (AAVM)

Rome, Italy, October 16 - 19 . 2018

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NINTH INTERNATIONAL CONFERENCE ON ANTIMICROBIAL AGENTS IN VETERINARY MEDICINE (AAVM)
Rome, Italy, October 16-19, 2018

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Prof. Stefan Soback

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TUESDAY, OCTOBER 16, 2018
16:00 Registration Opens
19:00 Welcome Reception

WEDNESDAY, OCTOBER 17, 2018
09:00 - 09:15 Opening Address

WELCOME AND INTRODUCTION
Petra Cagnardi, Conference Chairperson, Italy
Stefan Soback, Conference Past-Chairperson, Israel

09:15 - 10:45 Session 1: ANTIMICROBIAL RESISTANCE
Chairs: Peter Damborg, Denmark and Carl Cerniglia, USA

09:15 THE ROLE OF EFSA ON MONITORING AND RISK ASSESSMENT ACTIVITIES ON ANTIMICROBIAL RESISTANCE IN THE FOOD CHAIN
Ernesto Liebana, European Food Safety Authority (EFSA), Italy

09:45 ANTIMICROBIAL RESISTANCE AT THE FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAO)
April Johnson, Italy

10:15 ANTIMICROBIAL SUSCEPTIBILITY, RESISTANCE, LENGTH OF THERAPY AND CLINICAL OUTCOME: WHAT HAVE WE LEARNED FROM IN VITRO MEASUREMENTS?
Joseph Blondeau, Canada

10:45 Coffee Break and Poster Viewing

11:15 - 12:00 Session 1: ANTIMICROBIAL RESISTANCE (continued)
Chairs: Ernesto Liebana Criado, Italy and Nuno Silva, UK

11:15 FLUOROQUINOLONE RESISTANCE IN CLINICAL AVIAN PATHOGENIC ESCHERICHIA COLI (APEC) ISOLATES FROM FLANDERS (BELGIUM): IS THE POULTRY INDUSTRY AT RISK?
Robin Temmerman, Belgium

11:30 TRENDS OF RESISTANCE TO EXTENDED-SPECTRUM B-LACTAMS IN COMMENSAL AND CLINICAL ESCHERICHIA COLI FROM THE BROILER PRODUCTION PYRAMID
Ilias Apostolakos, Italy

11:45 THE EFFECT OF LACTOBACILLUS FERMENTUM ON THE ACTIVATION OF CHICKEN TLR4 AND DEFENSINS AFTER INFECTION BY C. COLI
Viera Karaffová, Slovakia

12:00 Lunch

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14:00	THE SHARED APPROACH OF THE ITALIAN AUTHORITIES FOR THE FIGHT AGAINST ANTIMICROBIAL RESISTANCE Stefania Delfrà, Italy Representative from the Italian Health Ministry
14:30	THE EFFECTS OF RESIDUE LEVELS OF TETRACYCLINE ON THE INTESTINAL MICROBIOME Carl Cerniglia, USA
15:00	Coffee Break and Poster Viewing
15:30 - 17:15	Session 1: ANTIMICROBIAL RESISTANCE (continued) Chairs: Jeffrey Watts, USA and Patrick De Backer, Belgium
15:30	THE HUMAN MEDICINE POINT OF VIEW Fatima M'Zali, France
16:00	ANTIMICROBIALS IN FARM ANIMALS: IMPACT ON THE ENVIRONMENT AND CONSEQUENT ANTIMICROBIAL RESISTANCE DISSEMINATION Petra Cagnardi, Italy
16:30	IN VITRO KILLING OF PASTEURELLA MULTOCIDA (PM) AND ACTINOBACILLUS PLEUROPNEUMONIAE(AP) BY CEFTIOFUR (CF), ENROFLOXACIN (ER), FORFENICOL (F), TILMICOSIN (TL) AND TULATHROMYCIN (TU) USING CLINICALLY RELEVANT DRUG CONCENTRATIONS Joseph Blondeau, Canada
16:45	ANTIMICROBIAL RESISTANCE IN OVINE BACTERIA: A SHEEP IN WOLF'S CLOTHING? Nuno Silva, UK
17:00	EXTENDED SPECTRUM B LACTAMASE-PRODUCING ENTEROBACTERIACEAE (ESBL-E) COLONIZATION IN RACE HORSES IN ONTARIO, CANADA Anat Shnaiderman Torban, Israel

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09:30	CLSI/VAST APPROACH FOR BREAKPOINTS DEFINITION Mark Papich, USA
10:00	ADMINISTRATION ROUTE AND DOSAGE OF SELECTED ANTIMICROBIALS IN PIGS: IMPACT ON GUT CONCENTRATION Mathias Devreese, Belgium
10:30	ANTIBIOTIC SUSCEPTIBILITY MONITORING OF VETERINARY PATHOGENS AND ZOONOTIC AND COMMENSAL ORGANISMS THROUGHOUT EUROPE – THE CEESA PROGRAMS Hilde Moyaert, Belgium
11:00	Coffee Break and Poster Viewing
11:30-12:30	Session 2: ANTIMICROBIAL SUSCEPTIBILITY TESTING (continued) Chairs: Pierre-Louis Toutain, UK and Mark Papich, USA
11:30	RAPID DETECTION OF TETRACYCLINE RESISTANCE IN BOVINE PASTEURELLA MULTOCIDA ISOLATES BY MALDI BIOTYPER ANTIBIOTIC SUSCEPTIBILITY TEST RAPID ASSAY (MBT-ASTRA) Laura Van Driessche, Belgium
11:45	CLINICAL BREAKPOINT OF DANOFLOXACIN AGAINST HAEMOPHILUS PARASUIS IN PIGS Haihong Hao, China
12:00	ANTIMICROBIAL SUSCEPTIBILITY OF MASTITIS-CAUSING BACTERIA FROM MILK FROM DUTCH DAIRY CATTLE Annet Heuvelink, The Netherlands
12:15	MONITORING AND STATISTICAL ANALYSIS OF ANTIMICROBIAL SUSCEPTIBILITY OF POULTRY PATHOGENS IN THE NETHERLANDS, 2014-2018 Jeanine Wiegel, The Netherlands
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14:00	MACROLIDES IN ANTIMICROBIAL SUSCEPTIBILITY TESTING OF SWINE RESPIRATORY PATHOGENS: ARE MIC VALUES OF NEWER MACROLIDES LIKE TILDIPIROSIN SIMILAR TO MIC VALUES OF OLDER MACROLIDES LIKE TILMICOSIN? Jobke van Hout, The Netherlands
14:15	NOVEL ANTIMICROBIAL FOR THE TREATMENT OF BOVINE MASTITIS Cathy Abberton, Ireland
14:30	STUDY OF CECAL MUCOSAL IMMUNE CELLS IN C. JEJUNI AND C. COLI CHALLENGE AFTER PREVENTIVE ADMINISTRATION OF PROBIOTIC STRAINS E. FAECIUM AL41 AND LACTOBACILLUS FERMENTUM CCM 7514 IN CHICKENS Viera Revajová, Slovakia
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15:00-16:15	Session 4: BIOSECURITY Chairs: Guido Di Martino, Italy and Mathias Devreese, Belgium
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15:30	FRIEND OR FOE? VERTICAL INTEGRATION AND SPREAD OF ANTIMICROBIAL RESISTANT ORGANISMS Helen Crabb, Australia
15:45	IS THE ANTIBIOTIC-FREE LABELLING HELPING THE FIGHT AGAINST ANTIBIOTIC RESISTANCE? Elisa Bianco, UK
16:00	LINKING ENVIRONMENTAL CONTAMINANT PCB ON TETRACYCLINE RESISTANCE IN ENTEROCOCCUS FAECALIS Yagmur Turgut, Turkey
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09:45	WHICH INTER-SPECIES DIFFERENCES SHOULD WE CONSIDER WHEN INTERPRETING AST DATA? Ronette Gehring, The Netherlands
10:15	PHARMACOKINETIC/PHARMACODYNAMIC ANALYSIS OF METRONIDAZOLE AND IMIPENEM USED TO TREAT BACTEROIDES SPP. INFECTION OF THE PLEURAL CAVITY IN HORSES Taisuke Kuroda, Japan
10:30	INTRAMUSCULAR ADMINISTRATION OF SODIUM BENZYLPENICILLIN WITH ADDITION OF LIDOCAINE AND ADRENALINE IN HORSES Lena Olsén, Sweden
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Antimicrobial resistance at the Food and Agriculture Organization of the United Nations (FAO).

April Johnson[1,*]

[1] *Animal Production and Health, Food and Agriculture Organization of the United Nations, Rome, Italy*

Abstract

The Food and Agriculture Organization of the United Nations (FAO) supports governments, producers, traders and other stakeholders to combat antimicrobial resistance (AMR) in line with Resolution 4/2015 which was adopted in 2015 at the FAO's thirty-ninth Conference on AMR, recognizing the threat AMR poses to public health and sustainable food production. Subsequently the FAO Action Plan on AMR, 2016-2020 was published outlining how the FAO supports the food and agriculture sectors to tackle AMR with a focus on four areas: 1) improving awareness on AMR and related threats, 2) developing capacity for surveillance and monitoring of AMR and AMU in food and agriculture, 3) strengthening governance related to AMU and AMR in food and agriculture and 4) promoting good practices in food and agricultural systems and the prudent use of antimicrobials. With donor support, FAO is working in over 25 countries to support development and implementation of National Action Plans (NAPs) on AMR. Additionally, FAO is developing tools and guidance that will help countries improve their AMR response such as the Assessment Tool for Laboratory and AMR Surveillance Systems (ATLASS). FAO works closely with the World Health Organization (WHO) and World Organization for Animal Health (OIE) as the Tripartite to combat AMR through implementation of the Global Action Plan on AMR (GAP), which has been endorsed by member countries of each of the organizations. The Tripartite has developed a national questionnaire on implementation of NAPs and is developing a Monitoring and Evaluation approach to track progress and impact of implementation of the GAP. The Tripartite is also developing a Development and Stewardship Framework to combat AMR, which guides the development, control, distribution and judicious use of new antimicrobial medicines, diagnostic tools, vaccines and other interventions, and is also designed to protect current medicines and promote affordable access. In addition, the Tripartite are members of the Secretariat of the ad hoc Interagency Coordination Group (IACG) on AMR, mandated by the UN General Assembly Resolution A/RES/71/3 to ensure sustained and effective global action to address AMR.

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Antimicrobial susceptibility, resistance, length of therapy and clinical outcome: what have we learned from in vitro measurements?

Joseph M. Blondeau[1,2*]

[1]*Clinical Microbiology, Royal University Hospital, Saskatoon, Canada,*
[2]*Clinical Microbiology, University of Saskatchewan, Saskatoon, Canada*

Abstract

Antibiotic activity is determined by measuring the minimum inhibitory concentration (MIC) against various species of bacteria. MICs that fall below achievable and sustainable therapeutic drug concentrations are considered susceptible. Mutant prevention concentration (MPC) testing determines the therapeutic drug concentration blocking growth of the least susceptible bacterial cell(s) present in high density (>107 colony forming units [cfu]) bacterial populations – such as those documented in acute infections. In vitro kill measurements have been used to differentiate antibiotics as being bactericidal or bacteriostatic and to determine the speed and completeness of reductions in viable cells in the presence of the drug(s) over time intervals. Recently published data reported on statistically significant differences between antibiotics in the speed and extent of bacterial killing at clinically relevant drug concentrations. The traditional definition of bactericidal and bacteriostatic may not apply when higher bacterial densities (>106 cfu/ml) are tested. In human medicine, in vitro microbiological measurements along with pharmacokinetic/pharmacodynamic modelling and clinical outcome studies have redefined the length of therapy (shorter durations) necessary for a number of clinical conditions and without compromising patient care. Shorter durations of therapy may also be possible for a number of infectious conditions in veterinary medicine. Shorter durations of therapy (where possible) are thought to reduce the likelihood for resistance selection, be more cost effective and be consistent with antimicrobial stewardship goals. This presentation will review what in vitro measurements (and their limitations) contribute to comparing antimicrobial agents and the likelihood such data influences length of therapy and clinical outcomes.

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How to lower antibiotic use in animals – the Danish approach.

Peter Damborg[1,*]

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

Abstract

Lowering use of antimicrobials in animals is a crucial step towards minimizing antimicrobial resistance. This presentation outlines some of the important actions implemented in Denmark to reduce antimicrobial consumption in animals. Importantly, these measures reflect an interplay between stakeholders representing national authorities, animal organizations, academia, and industry. One of the first key initiatives was the ban of the growth promoter avoparcin in 1995 following evidence of selection for vancomycin-resistant enterococci. Shortly thereafter, the national surveillance system DANMAP was started to monitor antimicrobial use and levels of antimicrobial resistance in humans and food animals. In 2000, the VetStat system was introduced, providing detailed data on all use of prescription medicines in production animals at farm-, species-, and age-level. These surveillance systems have since been a cornerstone in monitoring trends of antimicrobial usage and resistance, and they have provided scientific evidence for intervention measures such as the Yellow Card initiative in 2010. The Yellow Card initiative is used to evaluate and rank pig herds in a standardized way according to their antimicrobial use. If herds pass a predefined threshold, the authorities can issue an injunction requiring herd owners to lower their antimicrobial consumption. Recently, the Yellow Card initiative was updated with increased focus on critically important antimicrobials rather than total consumption only. Apart from legislative measures, the pig industry and the dairy cattle association have voluntarily ceased the use of cephalosporins since 2010 and 2014, respectively. Moreover, political targets have been set for reduction of antimicrobial usage, and treatment guidelines have been developed for rational antimicrobial use in pigs, cattle, horses, and pets. Taken together, these and other initiatives have reduced antimicrobial consumption across most of the veterinary sector in Denmark.

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Vespa

The effects of residue levels of tetracycline on the intestinal microbiome.

Carl Cerniglia[1,*]

[1] *US Food and Drug Administration, National Center for Toxicological Research, Jefferson, USA*

Abstract

The use of antimicrobials, such as tetracycline, in food-producing animals to treat, control and prevent disease may result in antimicrobial drug residues (ADR) in edible tissues from treated animals and contribute to the emergence of antibiotic resistant bacteria and impact the intestinal microbiome. The Veterinary International Conference on Harmonization (VICH) document (VICH GL36(R)/ FDA-CVM Guidance for Industry #159) provides guidance on evaluating the safety of veterinary ADR in the human foods as related to effects on the human intestinal microbiome. In the present study, we addressed data gaps regarding the effects of residual levels of tetracycline on binding to human feces, intestinal microbiome composition, antimicrobial resistance and gastrointestinal epithelial layer barrier disruption. High-performance liquid chromatography mass spectrometry assays showed that 25% (w/v) fecal slurries dosed with 0.15 and 1.5 µg/ml tetracycline had binding of 58.2±10.8% and 56.9±9.1%, respectively. Pyrosequencing results indicated that the effects of 0.15, 1.5, 15, and 150 µg/ml of tetracycline, after 24 h and 40 days of exposure were variable, with either no change or minor changes in total bacterial community. Among the 23 tetracycline resistance genes (TRGs) screened, four tet genes (tetO, Q, W, and X) were major TRGs in control and tetracycline-dosed fecal samples. A variable to slight increase of copy number of TRGs appeared to be related to tetracycline treatment, interindividual variability and duration of exposure. Intestinal epithelial cells (T84) treated at concentrations of 0.015, 0.15, 1.5, 15 and 150 µg/ml for 48 h in an in vitro cell culture model indicated that 15 and 150 µg/ml of tetracycline causes barrier disruption. Lower concentration levels showed little to no effect on epithelial cell integrity. This significant research provided data, scientific methodology and general recommendations to national regulatory authorities on tetracycline inactivation, bioavailability, barrier disruption, and antimicrobial resistance. The results of these investigations should improve the safety evaluation and risk assessment of antimicrobial residues and their impact on the gastrointestinal microbiota, with the goal of ensuring food safety.

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Antimicrobials in farm animals: impact on the environment and consequent antimicrobial resistance dissemination.

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Abstract

In intensive animal farming, antimicrobial drugs (AMD) are used for individual therapy, but also for mass medication involving use of large amounts of drugs. Some of the AMDs are eliminated as such and others as metabolites. Manure is commonly used for the fertilization of agricultural soils potentially resulting in an environmental load of drug residues. The environmental consequences from the soil fertilization with drug-contaminated manure represent a topic of increasing interest. The evaluation of the environmental risks deriving from the use of veterinary medicinal products is mandatory for all the new marketing authorizations in the EU. For the already authorized veterinary drugs this requirement does not exist. Thus, the real scenario of environmental exposure to AMDs remains incomplete. Besides soil and water contamination with undesirable substances, the persistence of veterinary AMDs in the environment may represent a toxicological risk for non-target species (e.g. fishes, plants, aquatic and terrestrial organisms). Additionally, an emerging concern is the occurrence and spread of antimicrobial resistance (AMR) in the environment. Along with its impact on the soil and water resistome, the distribution of drug-contaminated manure can affect the composition and functional properties of microbial communities or microbiome. The reservoir of resistance in the environment is a mix of naturally occurring resistance genes and those added via animal and human waste. Moreover, the selective effects of antimicrobials may have potential serious ecological and public health implications. Indeed, there is an increasing concern that environmental resistance may be transferred to humans. A research project has been granted by the Italian Ministry of School Education, University and Research (PRIN 2015KA3XFH) to evaluate the role of intensive animal farming (poultry, cattle and swine located in two Italian regions), as potential source of environmental antimicrobial contamination and resistance and as potential cause of toxic effects on non-target organisms. Analyses are carried out in order to generate data on antimicrobial concentrations, antimicrobial resistance genes (ARGs) and microbial community composition of animal manure and soil before and after the application of manure. The toxic effects on non-target organisms of the soil and freshwater compartments will be also investigated

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The EUCAST/VETCAST approach to breakpoint definition and determination.

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Abstract

VetCAST is the EUCAST (European Committee on Antimicrobial Susceptibility Testing) sub-committee for veterinary Antimicrobial Susceptibility Testing (AST). Its remit is both to define and harmonise clinical breakpoints (CBPs) for antimicrobial drugs (AMDs) used in veterinary medicine in Europe. This presentation outlines the procedures and reviews scientific options for solving challenges to the determination of specific CBPs for animal species, drug substances and disease conditions. VetCAST has adopted EUCAST approaches: the initial scientific step comprises data assessment; followed by procedures for decisions on the CBPs that include non-scientific considerations; and finally the release of recommendations for CBP implementation. The principal challenges facing VetCAST are those associated with the differing modalities of AMD administration, including mass medication, long-acting product formulations and local administration. Specific challenges comprise: breakpoint determination for mastitis treatment in dairy cattle; the range of species and within species breed considerations; and several additional variable factors not relevant in human medicine. These factors may prevent adoption of a single CBP covering all situations. Each CBP will be based on: (i) an epidemiological cut-off value (ECOFF) – the highest MIC that defines the upper end of the wild-type MIC distribution; (ii) a PK/PD breakpoint based on pre-clinical pharmacokinetic data [it is the highest possible MIC for which a given percentage of animals (e.g. 90%) in the target population achieves a critical value for the selected PK/PD index (fAUC/MIC or fT>MIC)]; and (iii) when possible, a clinical cut-off, which takes account of the relationship between MIC and clinical cure. For the latter, VetCAST acknowledges the paucity of such publicly available data in veterinary medicine. In consequence, the PK/PD breakpoint plays a pivotal role for VetCAST, as is similarly the case for EUCAST. When a CBP cannot be established, VetCAST will recommend use of ECOFF as a surrogate. For decision steps, VetCAST will follow EUCAST procedures involving transparency, consensus and independence. VetCAST will ensure free availability and widespread dissemination of information, concerning standards, guidelines, ECOFF, PK/PD breakpoints, CBPs and other relevant information for AST implementation. Finally, after establishing a CBP, VetCAST will promulgate widely expert comments and/or recommendations on CBPs to facilitate scientifically sound implementation in a clinical setting. VetCAST currently has 52 members and is currently completing its first clinical breakpoint definition (florfenicol in cattle against Pasteurella multocida and Mannheimia haemolytica).

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CLSI/VAST approach for breakpoints definition.

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Abstract

On June 28, 2018 the Clinical Laboratory Standards Institute (CLSI) Veterinary Antimicrobial Susceptibility Testing (VAST) subcommittee published the new standards for susceptibility testing (www.CLSI.org). These documents are the newest standards, representing over 20 years of developing susceptibility testing standards for pathogens isolated from animals. CLSI-VAST is the only organization in the world that has published breakpoints for animal pathogens. The current edition of the CLSI standard document has approximately 180 breakpoints for drug-bacteria combinations in the major veterinary species. There are now breakpoints for practically every approved antimicrobial agent for the label indication in veterinary medicine. CLSI-VAST is working to fill the gaps for the few agents that do not have clinical veterinary breakpoints. Because there are many human-labeled antimicrobial agents used in veterinary medicine, CLSI also has developed clinical breakpoints for these agents in the non-food animal species. CLSI-VAST develops the breakpoints through a rigorous process, using strict criteria. Sponsors are required to follow guidelines provided by CLSI and must submit data to support a proposed breakpoint. The data includes pharmacokinetic data in the target species, MIC distributions for the pathogens targeted, clinical data from the drug used under field conditions at the approved dose, and pharmacokinetic-pharmacodynamic (PK-PD) analysis, using Monte Carlo Simulations, to show that at the approved dose, the drug attains PK-PD targets for the labeled pathogen. For older agents that no longer have sponsors (generic drugs), a separate Working Group has developed guidelines to establish breakpoints for these agents. The Working Group uses PK-PD analysis, Monte Carlo simulations, MIC distribution data from laboratories, and published evidence of clinical experience. The CLSI uses a consensus-driven process. The consensus process involves the development and open review of documents, revision of documents in response to discussion, and, finally, the acceptance of a document as a consensus standard. All CLSI meetings are open to the public and all opinions are considered. When accepted, the CLSI breakpoints are regarded as a public standard, not a guideline. The standard documents developed through the consensus process identifies specific, essential requirements for materials, methods, or practices for use in an unmodified form. In order to follow the CLSI standard, the testing methods must be followed, including standardized media and test conditions. MIC data generated in media other than CLSI standard media (eg, Mueller-Hinton Broth), or alternative test conditions are not acceptable.

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Antibiotic susceptibility monitoring of veterinary pathogens and zoonotic and commensal organisms throughout Europe – the CEESA programs.

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Abstract

Antimicrobial resistance is a global concern for both animal and human health. Programs to monitor antibiotic susceptibility among veterinary pathogens as well as zoonotic and commensal bacteria are therefore essential. Various European countries have national monitoring programs in place for foodborne pathogens and commensal bacteria. The EFSA provides technical guidance and compiles the data from the Member States on antimicrobial resistance in zoonotic Salmonella spp., Campylobacter spp. and indicator Escherichia coli spp. from food-producing animals and retail meats. Only a few national surveillance programs monitor antibiotic susceptibility of target pathogens from farm and companion animals. The antibiotic susceptibility monitoring programs of CEESA are an ongoing collaboration among veterinary pharmaceutical companies since twenty years. CEESA conducts two types of monitoring: the EASSA program collects zoonotic and commensal bacteria at slaughter from healthy food-producing animals, and the target pathogen programs (VetPath, MycoPath and ComPath) collect bacterial isolates from diseased animals prior to antibiotic treatment. The latter programs are the only longstanding pan-European projects in veterinary medicine where antibiotic susceptibility data for a large variety of target pathogens are generated. Through valuable support by external laboratories and veterinary practitioners, CEESA has meanwhile generated a collection of >55,000 non-duplicate bacterial isolates. All CEESA projects apply uniform sample collection and bacterial identification to species level across EU Member States. A single central laboratory for each subprogram conducts quantitative antibiotic susceptibility testing to determine the Minimal Inhibitory Concentrations to a range of antibiotic compounds. Data are primarily used by member companies in registration and renewal dossiers but the programs also contribute to scientific research such as characterisation of ESBL/AmpC, mcr-colistin and qnr-quinolone resistance determinants. Results are disclosed through conferences and peer-reviewed publications. The standardised methodology of the CEESA programs makes these robust and valuable tools to address food safety concerns and to support responsible use of antibiotics in the field by giving the veterinarian information on resistance patterns in target pathogens

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Targeting bacterial virulence mechanisms for novel veterinary antibacterial therapies.

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Abstract

Over the past decade, the emergence of multi-drug resistant bacteria has limited the options available for the treatment of bacterial diseases in both human and veterinary patients. This evolving landscape has precipitated an urgent need for identifying alternative therapeutic targets without impacting the available approaches to treat infectious diseases in humans. In veterinary medicine, this need is further driven by the need for agents that prevent, control or treat animal diseases. This situation has been an incentive for developing agents in such alternative substrate categories as antimicrobial peptides, bacteriophages, microbiome modifiers, and immunomodulators. There has also been an increased interest in agents that can inhibit bacterial virulence mechanisms thereby limiting the disease-causing capabilities of the pathogen and allowing the host immune system to resolve the disease condition. Broadly, bacterial virulence mechanisms can be categorized into four types: tissue damaging toxins; host immune evasion mechanisms; adherence/protective structures; and immune function inhibitory toxins. Current research has been focused on strategies for interfering with each of these processes. However, the appropriate application of these agents, either as direct or adjunctive therapies, will vary between the various substrate categories. The purpose of this presentation is to discuss the various substrate categories and their applications as well as the issues (efficacy, safety) that need to be addressed to advance these types of agent into useful therapeutic entities.

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Biosecurity: structural and management measures to control diseases in poultry sector.

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Abstract

The EU Commission's Action Plan launched in 2011 against AMR contained 12 actions. Specifically, action n. 5 focuses on the prevention of diseases, reducing the use of antimicrobials and making sure they are used appropriately. At farm level, the implementation of adequate biosecurity procedures can be considered an effective measure to minimize the risk of disease outbreaks, making the need for antimicrobials less frequent. Biosecurity includes all measures to prevent the introduction of disease agents into a flock (external biosecurity) and to reduce the risk of transmission within the flock (internal biosecurity). It consists of three main components: isolation of the farm, movement control (of human, animal and materials), and sanitation protocols. A lack of biosecurity can contribute to the introduction and spread of disease between farms, within the same farm (e.g. between adjacent sheds) and from animals to humans (in the case of zoonoses). During the presentation an overview will be provided regarding major biosecurity structural and management features, within the specific framework of the poultry sector. This production system is particularly vulnerable to the pressure of environmental pathogens. Moreover, poultry industry is a strictly integrated system where affiliated farms are inevitably interconnected by vehicles (feed/animals supply, waste removal, etc.), people (workers, veterinarians, etc.) and geographical proximity within the same densely populated poultry area (i.e. premises are mostly assembled in few areas with a high density of poultry farms). Besides horizontal spread, also vertical transmission from breeders to eggs need to be taken into account (e.g. Salmonella, Mycoplasma). Therefore, a good biosecurity level should be combined with complementary strategies to improve health management, such as vaccinations, feed additives and feed formulations, genetic improvement of animals. The enhancement of health management along the poultry production chain (breeders, hatchery, meat farms) can be reflected by a decrement of antimicrobial usage in the last few years; to this end recent data collected at national level will be presented and discussed.

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Is there a connection of MIC and PK in veterinary therapeutics?

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Abstract

Minimum inhibitory concentration (MIC) is a quantitative antimicrobial test and presents an aspect on bacterial susceptibility considering clinical resistance breakpoints published by EUCAST, VetCAST and CLSI. Resistance breakpoints are closely related to achievable drug concentrations in target tissues. PK features, such as serum concentration over time and area under the serum concentration-time curve (AUC), when integrated with MIC values, can theoretically predict the probability of bacterial eradication and clinical success. The key point is that can resistant bacteria be inhibited considering data provided from in vitro studies including MIC tests, MPCs and PAE/PA-SME without performing in vivo PK tests, simultaneously? The study of PD is central for the optimization of an antimicrobial therapy. In vitro models can provide important information about the time course of an antimicrobial effect, which can be used in the dose-response relationship, and to determine PK/PD target measures that are predictive of clinical efficacy. Previous in vitro dose optimization studies showed that not only MIC results but also the genetic mechanisms of resistance are determinative for the bactericidal activity of antimicrobials against resistant bacteria. Therefore, in addition to MIC assays, genetic mechanism-based PD models can be used to provide accurate prediction of drug's effects on resistant bacteria. Another key point is that is PK profile of a licensed antimicrobial in an animal having healthy biotransformation functions compatible with likely changed bactericidal activity of the drug due to the genetic mechanism of resistance? Antibiotic resistance is an important public health issue, and high risk for the treatment of infectious diseases can arise from recommended available dosage regimens. In cases involving a lack of effective agents, combinations of two or more antibiotics are often used in the hopes of achieving a synergistic effect. Bactericidal activity of an antimicrobial in the combination may be different from its individual bactericidal activity. Therefore, it can be assumed that known PK profiles of antimicrobials cannot be correlated with in vitro PD profiles of drugs or dose determination considering known PK profiles may not be sufficient to achieve bacterial eradication. Acknowledgements: TUBITAK (TOVAG-214O316).

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Which inter-species differences should we consider when interpreting AST data?

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Abstract

Classification of a bacterial isolate as sensitive or resistant rests on the assumption that appropriate concentrations of the antimicrobial agent will be achieved at the site of infection when registered label dosage regimens (or a generally accepted extralabel dosages) are used. If dosages are not adjusted for inter-species pharmacokinetic differences, treatment may fail due to lower than expected concentrations at the site of infection. There are many pathophysiological factors that differ within and between species that could alter the pharmacokinetics of an antimicrobial agent. These factors include differences in diet, gastrointestinal anatomy and function, plasma protein binding, blood flow to organs, pH in different tissue compartments, and reproductive and lactational status, all of which can alter the rate and extent of absorption, distribution or elimination of an antimicrobial agent. This talk will review what is known about the effect of these factors on the pharmacokinetics of commonly used veterinary antimicrobial agents and discuss how these should be considered when interpreting AST results for different animal species.

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Technological tools for infection detection.

Dries Berckmans[1,*], Wim Buyens[2], Zhao Ying Cui[3]

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Abstract

For economic reasons, the number of animals per farm(er) has increased drastically over the last decades and will continue to do so in the future. Due to this trend, it has become impossible for farmers and veterinarians to observe large herds intensively on a frequent basis. However, fast detection and identification of infections remains extremely important for the economic result of the growth cycle, animal welfare and reduction of the overall use of antibiotics. These challenges are the driving force behind the continuous growth of the precision livestock farming sector where advanced technologies are used to optimize the contribution of each animal. Featured in the top 10 most innovative companies of Belgium, SoundTalks is a spin-off company of the KULeuven and the University of Milan that was founded in 2011. The company delivers benchmarking work in the field of sound analysis for livestock health applications. Data collection has become a commodity nowadays but recording of sound in livestock facilities still poses many challenges. Our current commercially available product is an early warning tool for the automated detection of respiratory problems in fattening pig houses. Based on the continuous processing of the sounds recorded in a pig barn, SMS or e-mail notifications are sent from the SoundTalks cloud to farmers and veterinarians. Different types of users will receive the information in different formats to meet their specific needs such that it can assist them in the best possible way during their daily routine. Early warning and appropriate action can improve the productivity of each animal and lower the amount of antibiotics used. This presentation will introduce the company and its work in precision livestock farming. It will mention the challenges associated with the collection and processing of data from livestock facilities and provide possible solutions. Lastly it will show how data is translated into customized information by automated analysis.

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Fluoroquinolone resistance in clinical Avian Pathogenic Escherichia Coli (APEC) isolates from Flanders (Belgium): is the poultry industry at risk?

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Abstract

Colibacillosis is one of the leading causes of disease-related economic loss in the poultry sector. Fluoroquinolones are frequently used antimicrobials for the treatment of APEC infections in Europe and Asia. However, rapid development and selection of resistance to this class of antimicrobial drugs is a significant problem. The objective was to investigate the occurrence of antimicrobial resistance against enrofloxacin in APEC strains in Flanders by determining the minimum inhibitory concentrations (MIC) and the mutant prevention concentrations (MPC) and by characterizing resistance genes through PCR, gel electrophoresis and gene sequencing. 126 APEC strains from broilers with clinical colibacillosis were obtained via Animal Health Care Flanders (DGZ) and Sciensano (November – June 2018). Slide agglutination was used to test for O1, O2 and O78 antigens. The MIC was determined using a commercial gradient strip test (Liofilchem s.r.l., Roseto degli Abruzzi, Italy). The MPC was determined through the agar dilution method on the clinically susceptible (MIC ≤ 0.25 µg/mL) and the clinically intermediate (0.5 µg/mL ≤ MIC ≤ 1 µg/mL) strains. Ninety-six of the 126 strains were serotyped so far and the MIC of these strains was determined. O78 was the most prevalent serotype (17%). The majority of the strains (69%) could not be identified. Fourty-three percent of the strains were non-wild type (ECOFF: 0.125 µg/mL), 23% were clinically intermediate and 11% were clinically resistant (MIC ≥ 2 µg/mL). The MPC values of the clinically susceptible strains ranged from 0.25 µg/mL to 2 µg/mL. Some strains with low MIC values (e.g. 0.016 µg/mL) had rather high MPC values (e.g. 1µg/mL), thus portraying a large mutant selection window (MSW). The MPC values of the clinically intermediate strains ranged from 1 µg/mL to 32 µg/mL. The remainder of the strains (n=30) will be serotyped and MIC and MPC values will be determined. From all the non-wild type strains, the presence of plasmid-associated resistance genes (qnrS, A, B) and the sequence of chromosomal resistance genes (gyrA, parC) will be evaluated as well. These results will be presented at the conference. Acknowledgements: The authors would like to thank Sciensano for the supply of the APEC strains. This research is supported by the Special Research Fund of Ghent University grant number BOF17/STA/014.

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Trends of resistance to extended-spectrum β-lactams in commensal and clinical Escherichia coli from the broiler production pyramid.

Ilias Apostolakos[1,*], Luca Fasolato[1], Matteo Cuccato[1], Jacopo Ferraresso[1], Roberto Rizzo[1], Massimo Zago[1], Alessandra Piccirillo[1]

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Abstract

The presence of extended-spectrum and AmpC β-lactamase-producing *Escherichia coli* (ESBL/AmpC-EC) in food-producing animals can negatively affect human and animal health. This study aimed to determine the occurrence of ESBL/AmpC-EC in the whole broiler production pyramid in Italy by sampling three production chains. For each chain, we sampled Parent Stock (PS) chickens at the age of one-day and during the laying period. The offspring of the sampled PS was tracked and sampled in four commercial broiler farms during the first week of life and during the last week before slaughter. Carcasses of these broilers at the slaughterhouse and tissue swabs from colibacillosis cases were also collected. All samples (cloacal swabs from chickens and rinsates from carcasses) were processed on both Eosin Methylene Blue agar (EMB) and EMB supplemented with 1mg/L cefotaxime (CTX-EMB) except for tissue samples, which were analysed only on EMB. Three confirmed *E. coli* colonies (2 from EMB and 1 from CTX-EMB) were subjected to disk-diffusion test for ESBL/AmpC-production according to CLSI guidelines. Detection rates using CTX-EMB demonstrated the presence of ESBL/AmpC-EC at all levels of the broiler production pyramid. Extremely-high percentages (92.5% on average) were encountered in one-day-old PS chickens, which dropped significantly to moderate ratios (20% on average) during the laying period. Persistent occurrence of ESBL/AmpC-EC was found in broiler farms at both sampling times with an average detection rate of 64.2% (range 25-100%) for day-old broilers to 42.9% before slaughter (range 15-95%). Also at the bottom of the production pyramid, carcasses were highly contaminated with ESBL/AmpC-EC (51.3% on average, range 15-75%). Conversely, detection rates of ESBL/AmpC-EC with EMB were substantially lower. The proportion of positive samples was seven times lower on average compared to CTX-EMB and for 14 out of 42 sampled flocks, no samples were found positive on EMB. Additionally, only 6.6% (10/152) of clinical *E. coli* showed phenotypic ESBL/AmpC-production. This finding suggests that, although present, ESBL/AmpC-EC colonise the broiler gut at low levels.

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The effect of lactobacillus fermentum on the activation of chicken TLR4 and defensins after infection by *C. coli*.

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Abstract

Campylobacter jejuni and *C. coli* are identified as major causes of food-borne disease in humans worldwide. Chicken TLRs are important in the recognition of PAMPs to induce the production of antimicrobial peptides and the expression of co-stimulatory molecules that may initiate adaptive immunity responses. Beta-defensins are antimicrobial peptides, which represent one family of host defence peptides that are modulated during *campylobacter* infection. On the other hand, it has been reported that campylobacter usually significantly downregulated their production. Promising therapy appears to be use of probiotic strains such as *Lactobacillus* spp. The aim of this study was to determine whether probiotic-feeding affected the expression of TLR4 and selected defensins (AvBD2, AvBD6) in response to *C. coli* challenge in the cecum of broilers. Forty one day-old broilers were randomly divided into 4 experimental groups (n= 10): control (C), *L. fermentum* CCM7514 (LB), *C. coli* (CJ), and combined *L. fermentum* CCM7514 + C. coli (LBCJ). *L. fermentum* was administered daily per os to LB and LBCJ groups from day 1 to day 7 of the experiment. *C. coli* was administered orally on day 4 of the experiment by a single dose to CJ and LBCJ groups. Samples from cecum were collected on day 4 and 7 post- *Campylobacter* infection (dpi). Samples were homogenized and total of RNA was isolated. Specific primers were used. Amplification and detection of specific products were performed using CFX 96 RT system with predefined program. Relative mRNA expression of defensins was upregulated in combined group compared to other groups ($P<0.001$) at 7 dpi. However, the increase relative expression of TLR4 was recorded in LB group compare to other groups ($P < 0.05$; $P < 0.01$; $P<0.001$) during both samplings. These results suggest that probiotics-feeding may stimulate the immunodefence system mediated by defensins and TLR4 against infection by *C. coli* in the cecum of broilers.

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In vitro killing of *Pasteurella Multocida* (PM) and *Actinobacillus Pleuropneumoniae* (Ap) by Ceftiofur (CF), Enrofloxacin (ER), Forfenicol (F), Tilmicosin (TL) and Tulathromycin (TU) using clinically relevant drug concentrations.

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Abstract

Bacterial killing is both important and necessary for recovery from an infectious disease and antimicrobial agents are important for inhibiting (bacteriostatic) or killing (bactericidal) pathogenic bacteria. The minimum inhibitory concentration (MIC) is used to determine, in vitro, susceptibility or resistance of bacteria to antibiotics and the mutant prevention concentration (MPC) determines the antimicrobial drug concentration blocking growth of the least susceptible bacterial cell present in high density bacterial populations, such as those seen during infection. Neither MIC or MPC is a measure for bacterial killing. We determined the killing of strains of PM (n=3) and AP (n=3) by 5 drugs using the MIC, MPC and maximum serum (Cmax) drug concentrations (DC). MIC/MPC testing utilized 100000 or 1 billion colony forming units (CFU/ml) against doubling drug dilutions. The lowest DC preventing growth (24/48 hours incubation at 35-37° C, ambient air) is the MIC/MPC. For killing, 100000 CFU/ml was exposed to the MIC, MPC and Cmax DC and aliquots sampled at 5, 10, 15, 20, 25, 30, 60, 120 and 180 minutes (M) after drug exposure. The log10 (LT) and percent kill (%) in viable organisms were recorded. MIC DC was poor at killing PM and AP. For PM at the MPC DC 89% (1LT) of cells were killed by ER at 120 M versus 17-36% (0.1-0.6 LT) kill for CF,F, TL and TU; killing increase to 96% (1.5LT) for ER by 180 M versus 28-57% (0.1-1.1 LT) for the other drugs. At the Cmax DC, 99% (2.5LT) of bacteria were killed by ER at 120 M versus growth (G) to 82% (CF) (G-1.7LT) kill for the other drugs. For AP at the MPC DC, 98% (2.6LT) of cells were killed by ER by 60 minutes versus G-67% (G-1.9LT) kill for the other drugs: >99% (3.5LT) for ER by 120 M versus 14-68% (.2-2.9LT) for the other drugs. At the Cmax DC, 93% (1.3LT) of cells were killed ER at 20 minutes versus G-64% (CF) (G-0.1-0.5LT) for the other drugs; 99% (2.1LT) for ER at 60 M versus G-88% (CF) (G-0.8LT) for the other drugs. At MPC and Cmax DC, ER killed PM and AP bacterial cells more rapidly and more completely than did the other drugs tested. Such finding may be important for minimizing resistance selection, choosing an antibiotic for treatment and along with clinical outcome data, length of therapy. Rapid killing of bacteria may reduce resistance selection.

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Antimicrobial resistance in ovine bacteria: a sheep in wolf's clothing?

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Abstract

To monitor the prevalence of antimicrobial resistance (AMR), methods for interpretation of antimicrobial susceptibility phenotypes of bacteria are needed. Reference limits to declare resistance are generally based on data obtained from human bacterial isolates but may not be appropriate for bacteria from livestock or other animals. In this study, the observed prevalence of AMR was compared between standard and bespoke interpretations based on clinical breakpoints or epidemiological cut-offs (ECOFF) using gram-positive (Staphyhlococcus aureus) and gram-negative (Escherichia coli) bacteria from sheep as exemplar. The sheep bacterial isolates were obtained from a cross-sectional study on three farms in Scotland and a longitudinal study of a single sheep flock in Norway between 2016 and 2017, whereby S. aureus was predominantly obtained from milk or mammary glands whilst E. coli was mostly obtained from faecal samples. Disc diffusion testing was used to determine inhibition zone diameters, which were interpreted using clinical breakpoints or ECOFFS as provided by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) or the Clinical & Laboratory Standards Institute (CLSI). Prevalence of AMR was low based on clinical breakpoints, e.g. only 3.8% for penicillin resistance in S. aureus, but EUCAST-recommended ECOFF values often bisected normal distributions of observed inhibition zone diameters, e.g. for cephalosporin or carbapenem resistance in E. coli. Thus, the use of EUCAST-recommended reference data could lead to erroneous reporting of high prevalence of resistance among livestock isolates. Using the Normalized Resistance Interpretation method, new ECOFF values were established to distinguish wild-type (WT) from non-wild type (NWT) populations and to recalculate the prevalence of AMR. Our data indicate that the livestock industry should establish and seek recognition for bespoke cut-off values for AMR monitoring in bacterial isolates derived from sheep to avoid inappropriate use of cut-offs from human medicine. The latter could lead to misclassification and high apparent resistance rates, suggesting an AMR problem that may not actually exist.

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Extended spectrum β lactamase-producing Enterobacteriaceae (ESBL-E) colonization in race horses in Ontario, Canada.

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Abstract

Antibiotic resistance is a global problem with a complex epidemiology. We aimed to investigate the prevalence, molecular epidemiology and risk factors for ESBL-E colonization in race-horses, since this unique equine population is found under intensive management, training and medical care. A prospective cross-sectional study was performed involving fecal samples collected from thoroughbred horses that were housed at a Woodbine racetrack in Ontario, Canada. Samples were enriched in Luria-Bertani broth, plated onto CHROMagarESBL plates, and sub-cultured to obtain pure cultures. ESBL production was confirmed using combination disc assay. Bacterial species were identified via MALDI-TOF and antibiotic susceptibility profiles were assessed using Vitek-2. E. coli sequence types were determined using Multi Locus Sequence Type (MLST) analysis. Medical records were reviewed and assessment of risk for individual variables was performed (SPSS). Overall, 169 adult thoroughbred horses, originating from 16 different barns, were sampled. ESBL-E colonization rate was 12% (n=21/169); 22 isolates ESBL-E were molecularly studied (one horses had two isolates). The main species was E. coli (91%) and the major ESBL gene group was CTX-M-1 (59%). Other ESBL-E species were Proteus hauseri and Enterobacter cloacae (one isolate each). Nine different E. coli sequence types were identified: ST1730 (in 2 horses from different barns), ST10 (in 4 horses, from 3 different barns), ST1250 (in 3 horses, from 3 different barns), ST1403 (in 3 horses, from one barn), and five single isolates sequence types- ST1462, ST4527, ST7870, ST2008, and ST86. Two new E. coli sequence types were identified. Sixty-four percent of total isolates were defined as multidrug resistant. Resistance rates to antibiotics of ESBL-E were 71% for trimethoprim-sulfa, 62% for tetracycline, 62% for gentamicin and no resistance was identified for quinolones, amikacin and carbapenems. ESBL-E colonized horses originated from 8/16 different barns; whereas 48% (10/21) of them originated from one specific barn. Overall, antibiotic treatment in the previous month was found as a risk factor for ESBL-E colonization (P<0.05). Our findings demonstrate the potential diverse reservoir of ESBL-E in race-horses. Multidrug resistant bacteria should be further investigated to improve antibiotic treatment regimens and equine welfare.

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Rapid detection of tetracycline resistance in bovine Pasteurella multocida isolates by MALDI BIOTYPER antibiotic susceptibility test rapid assay (MBT-ASTRA).

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Abstract

Pasteurella multocida is best known for its role in infectious bronchopneumonia, a disease which has a major economic and animal welfare impact in cattle production systems worldwide. Additionally, it is the leading indication for antimicrobial use in calves. To rationalize antimicrobial use in food producing animals, availability of a rapid and reliable antimicrobial susceptibility test is imperative. MALDI Biotyper antibiotic susceptibility test rapid assay (MBT-ASTRA) is a matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS)-based approach for rapid susceptibility testing. The objective of the present study was to design an MBT-ASTRA procedure for resistance detection in P. multocida for tetracycline, a frequently used antimicrobial for this indication. On 100 clinical isolates antimicrobial susceptibility for tetracycline was determined with MIC-gradient strip test, disk diffusion and MBT-ASTRA. The optimal MBT-ASTRA procedure was performed using CAMHB (Cation-adjusted Mueller Hinton broth) medium, with a concentration of 4 µg/mL tetracycline, a starting concentration of 1.5x10^7CFU/mL of bacteria and a cutoff value of the relative growth ratio of 0.5. Sensitivity and specificity of the MBT-ASTRA procedure were 95.7% and 100% respectively, classifying 98% of the isolates correctly after only three hours of incubation. Sensitivity and specificity of disk diffusion were 93.5% and 96.3%, respectively, classifying 95% of the isolates correctly. In conclusion, the MBT-ASTRA method shows all the potential to fulfil the need for rapid and reliable tetracycline susceptibility testing in P. multocida. This new technique could allow the rationalization of antimicrobial use in outbreaks of bronchopneumonia in cattle, and potentially many other clinical presentations across species.

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Clinical breakpoint of danofloxacin against Haemophilus parasuis in pigs.

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Abstract

Establishment of clinical breakpoint (CB) of danofloxacin against H. parasuis is of great significance for antimicrobial susceptibility testing and effective treatment of H. parasuis with minimizing development of fluroquinolone resistance. The epidemiological cut off value (ECOFF) or wide type cut off (COWT) was developed based on MIC distribution of 143 H. parasuis collected from diseased animals and calculated using Ecoffinder recommended by EUCAST. The pharmacodynamic cutoff (COPD) was established base on the pharmacokinetic (PK)-pharmacodynamic (PD) modeling of danofloxacin both in plasma and pulmonary epithelial lining fluid (PELF) in pigs. The PK-PD target was set by Hill formula and Ex-vivo time killing curve. The COPD was analyzed by Monte Carlo analysis using Crystal Ball. Five repetitive MICs and dose regimen designed from PK-PD modeling were selected for clinical trials. The clinical cutoff (COCL) was established based on the relationship between possibility of cure (POC) and MIC using "WindoW" approach, nonlinear regression and CART analysis. The CB was determined by the CB decision tree recommended by CLSI. Our results showed that the MIC50 and MIC90 for danofloxacin against 143 H. parasuis were 4 μg/mL and 16 μg/mL, respectively. The ECOFF value was calculated as 16 μg/mL based on the MIC distribution. From the concentration-time curve of danofloxacin, a two-compartment model was used for PK analysis. The PK parameters, including AUC, Cmax, Tmax, in plasma and PELF were 4.47 ± 0.51 h·μg/mL and 24.28 ± 2.70 h·μg/mL, respectively. The COPD in plasma and PELF were 0.125 μg/mL and 0.5 μg/mL, respectively. The COCL analyzed by the WindoW approach, nonlinear regression and CART analysis were 0.125~4 μg/mL, 0.428 μg/mL and 0.56 μg/mL, respectively. The eligible COCL was 0.25 μg/mL. The CB based on PK-PD model in plasma and PELF was 0.25μg/mL and 16 μg/mL, respectively. In conclusion, the CB of danofloxacin against H. parasuis were established for the first time, providing criterion for detection of danofloxacin-resistant H. parasuis and reference for effective treatment of clinical infection caused by H. parasuis.

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Antimicrobial susceptibility of mastitis-causing bacteria from milk from Dutch dairy cattle.

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Abstract

The objective of this study was to analyse the in-vitro antimicrobial susceptibility of mastitis-causing bacteria from milk from Dutch dairy cattle. We focused on Staphylococcus aureus, coagulase-negative staphylococci (CNS), Streptococcus uberis, Streptococcus dysgalactiae, Streptococcus agalactiae, Escherichia coli, and Klebsiella spp. Isolates originated from routine milk submissions to the laboratory of GD Animal Health, in 2015-2017. Minimal inhibitory concentrations (MICs) of antimicrobials were assessed by broth microdilution following CLSI recommendations. Panels with antimicrobials commonly used in the Netherlands to treat mastitis-causing bacteria were evaluated. MICs were interpreted as susceptible, intermediate and resistant based on CLSI human-derived clinical breakpoints (when available) because no bovine-specific breakpoints are available. Additionally, MIC50 and MIC90 values were calculated. For each bacterial species one randomly selected isolate per farm per year was included, resulting in MIC results for 6,525 isolates. S. aureus (n=1,294) were susceptible for most antibiotics tested, except for penicillin-G, with resistance percentages varying from 5.4-7.2% of isolates per year. Less susceptibility was seen among CNS (n=1,027); yearly 18.4-27.7% of isolates were resistant to penicillin-G, 22.4-25.4% to oxacillin, and 5.4-10.3% to erythromycin. Staphylococcus haemolyticus (n=250), Staphylococcus epidermidis (n=182), Staphylococcus chromogenes (n=174), Staphylococcus sciuri (n=70), Staphylococcus equorum (n=67), and Staphylococcus simulans (n=62) showed significantly different phenotypical antimicrobial susceptibility profiles. Among S. uberis (n=1,268) and S. dysgalactiae (n=695) yearly resistance percentages for erythromycin were 7.1-11.7% and 3.6-8.3%, respectively. And for 23.4-28.4% and 8.8-11.1% of isolates, respectively, elevated MICs for clindamycin were found. The number of S. agalactiae (n=24) isolates was too low for further analysis. Among E. coli (n=1,926), highest resistance percentages were seen for ampicillin, trimethoprim/sulfamethoxazole, and streptomycin: 8.4-12.3%, 8.6-10.2%, and 9.6-12.6%, per year, respectively. Resistance among Klebsiella spp. (n=291) was low, with the percentage of streptomycin resistance isolates being the highest, varying from 7.6-12.0% per year. In conclusion, mastitis-causing bacteria from milk from Dutch dairy cattle are susceptible to most commonly used antimicrobials in the Netherlands for treating mastitis. Different CNS species show different antimicrobial susceptibility patterns. Identifying CNS at species level and analysing results per species is relevant for monitoring purposes, and may also have implications for approaching mastitis problems in the field.

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Monitoring and statistical analysis of antimicrobial susceptibility of poultry pathogens in The Netherlands, 2014-2018.

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Abstract

The use of antibiotics in Dutch poultry has been reduced greatly, i.e. 74% reduction compared to the use in 2009 in broilers. To continue this prudent use of antibiotics, correct use and information of resistance of pathogens is essential. Antimicrobial susceptibility (AMS) patterns of pathogenic bacteria can be of great use for veterinarians in choosing a treatment for diseased flocks. Knowledge on the dynamics of resistance can be of influence on policy concerning antibiotic use. Isolates of Escherichia coli, Enterococcus species and Staphylococcus aureus from diseased chicken flocks were collected in the period from October 2014 to May 2018. Minimal inhibitory concentrations (MIC) of 18 and 12 commonly used antimicrobial agents (AM's) were assessed for E.coli and both cocci, respectively, and MIC50 and MIC90 values were determined. Veterinary breakpoints (when available) were used to indicate whether an isolate was considered susceptible, intermediate or resistant. Additional information on origin of the isolates was collected, such as type of bird (meat or layer and end product, rearing or reproduction), age, treatment history and sampling location in the body. Uni- and multivariable analysis (for 9 AM's) were performed to investigate associations between the features describing the origin of the isolates and the probability of an isolate being resistant. 1,707 E.coli isolates were used for the multivariable analysis. Results of AMS patterns are provided for E. coli, Enterococcus cecorum and S. aureus. Univariable analysis showed a difference in percentage of resistant isolates over the years and between the isolates from broiler and layer type birds. Multivariable analysis results varied between antimicrobials. Isolates from layer type birds resulted in lower odds for resistance (in 5 out of 9 AM's), rearing also resulted in lower odds (3 out of 9). The effects of treatment history, year of isolation and sampling location were ambiguous, resulting in either higher or lower odds, depending on the AM. The effect of age on resistance appeared to be non-linear, however 7 days old or younger resulted in lower odds for resistance (7 out of 9). There is a great need for establishment of specific clinical breakpoints for poultry to allow correct and internationally accepted interpretation of AMS to resistance levels. However, by using quantitative data, like MIC, it is possible to analyze trends and developments in AMS. The results of this multivariable analysis provide leads for further research into the dynamics of resistance in poultry pathogens.

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Macrolides in antimicrobial susceptibility testing of swine respiratory pathogens: are MIC values of newer macrolides like tildipirosin similar to MIC values of older macrolides like tilmicosin?

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Abstract

The objective was to evaluate whether MIC values of tildipirosin and tilmicosine are similar for Actinobacillus pleuropneumoniae (APP), Bordetella bronchiseptica (BBS), Pasteurella multocida (PMU) isolates from diseased pigs. Simultaneously with MIC determination, disk diffusion was done. APP, BBS, PMU were cultured from samples from pigs, submitted to GD Animal Health (GD AH). Laboratory tests were conducted at GD AH, results were interpreted according to CLSI standard procedures for testing susceptibility of veterinary pathogens. In total 79 APP, 71 BBS, 106 PMU isolates were cultured. For 95% (n=75) of APP, 54% (n=38) of BBS and 87% (n=89) of PMU isolates, MIC values of tilmicosin and tildipirosin differed more than one dilution step. Overall, for 99% (n=200) of these isolates tilmicosin MIC values were two or more dilution steps higher than of tildipirosin. All APP isolates tested were susceptible for tildipirosin, both by broth microdilution (n=79) and agar diffusion (n=77). 96% (n=76) of APP isolates were resistant to tilmicosin by broth microdilution, 35% (n=27) were resistant by agar diffusion. Based on MIC values, 54% (n=38) of BBS isolates were resistant to tildipirosin; all isolates were susceptible for tildipirosin by agar diffusion ($p<0.01$). No CLSI breakpoints for the combination BBS and tilmicosin were available. For PMU isolates, overall percentages susceptible/resistant to tilmicosin and tildipirosin did not differ significantly for both broth microdilution (n=102) and agar diffusion (n=105). Almost all PMU isolates were susceptible for both tilmicosin and tildipirosin; 8% (n=8) were resistant for tilmicosin by broth microdilution. 93% (n=95) of individual PMU isolates had similar results (susceptible/resistant) for both tilmicosin and tildipirosin, based on MIC values; 99.0 % (n=104) had similar results by agar diffusion. In conclusion, tilmicosin can be used to evaluate susceptibility/resistance of PMU to both tilmicosin and tildipirosin, both by broth microdilution and agar diffusion. In case of testing the susceptibility of APP according to CLSI standard procedures (incubating plates in enriched CO_2 atmosphere) it is recommended to control the pH of the medium to be able to use tilmicosin as indicator of tildipirosin susceptibility. Due to absence of clinical veterinary breakpoints, for BBS no clear conclusions can be drawn.

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DIPARTIMENTO DI SCIENZE VETERINARIE PER LA SALUTE, LA PRODUZIONE ANIMALE E LA SICUREZZA ALIMENTARE
Vespa

Novel antimicrobial for the treatment of bovine mastitis.

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Abstract

The occurrence of antibiotic-resistant bacteria is an increasingly prevalent societal issue globally. The fact that many antibiotics are no longer effective against bacteria is of particular concern in the veterinary sector, the largest consumer of antibiotics. Bovine mastitis is the most critical infectious disease affecting the dairy industry, leading to recurrent treatment failures, long periods of poor milk quality, loss of income to farmers, and, in many cases, the premature culling of animals. Due to the sheer number of causative organisms and their ubiquitous presence, mastitis eradication is unattainable. Westway Health is currently developing a novel treatment for bovine mastitis in lactating cows, based on the production of hypoiodite, an antimicrobial agent that can be produced by the reaction of peroxide in the presence of iodide. Due to the nature of this antimicrobial therapy, no antibiotic residues remain in the milk; therefore, little-to-no withdrawal period is required and the milk can be added to the bulk tank during treatment. Antimicrobial activity was tested, in vitro, against a panel of mastitis isolates (including E. coli, S. aureus, and Strep. spp). Minimum inhibitory concentrations (MICs) were determined to be comparable to many antibiotic treatments, and time-kill assays demonstrated an equivalent kill profile to a product currently on the market. Induction of resistance to hypoiodite was attempted over 12 days; E. coli developed resistance to all antibiotics tested within the experimental time frame, yet no resistance developed against hypoiodite. A model-udder system was designed to examine dispersal and dosage of the antimicrobial as the udder fills with milk; and subsequently, cows were identified for in vivo clinical trials based on high somatic cell counts (SCC) and the presence of clinical mastitis. Trials have been conducted on a multitude of farms across Europe, where SCCs and iodine residues have been measured over time and bacterial analysis has been carried out. The treatment was well tolerated by the cows and individual SCCs decreased in response to treatment, associated with clinical and bacteriological cures. Long-term follow-up of the animals indicated no adverse effects. We're now working towards regulatory approval of a novel mastitis treatment.

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Study of cecal mucosal immune cells in *C. jejuni* and *C. coli* challenge after preventive administration of probiotic strains *E. faecium* AL41 and Lactobacillus fermentum CCM 7514 in chickens.

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Abstract

Within the EU, campylobacteriosis is a frequently reported zoonotic disease in humans. Campylobacter species are especially associated with human gastrointestinal diseases. *C. jejuni* and *c. coli* occurring in chickens are responsible for them. The intestinal microbiota are maintained in a dynamic relationship with the host, involving a complex 'trialogue' between microorganism, epithelium and mucosal immune system. Noncommensal and probiotic bacteria are able to induce a gut mucosal immune response. The mechanisms by which probiotic bacteria affect the immune system are attributed to an increase in the innate or acquired immune response. We studied by flow cytometry in chickens the cecal intraepithelial and lamina propria CD3+, CD4+, CD8+ and IgA+ lymphocyte subpopulations in *C. jejuni* and C. coli infection after preventive administration of probiotic strains *E. faecium* AL41 and Lactobacillus fermentum CCM 7514. Two experiments with different probiotic strains and campylobacter bacteria were done in one-day-old broiler chickens of COOB 500 hybrid. Seven days preventive administration of *Enterococcus faecium* AL41 and C. jejuni (108 CFU/0.2 ml PBS) challenge on day 4 were used as first, Lactobacillus fermentum CCM 7514 and C. coli as second. Individual perorally administration of bacteria were used in campylobacter (CJ, Cc), probiotic (EF, LB) and combine groups (EFCJ, LBCc) compared to negative group without any bacteria in two samplings on days 4 and 7 post infection (dpi). Isolated cecal IEL and LPL, flow cytometry, direct immunofluorescence and double staining of lymphocytes by labelled mouse anti-chicken monoclonal antibodies were used. *Enterococcus faecium* AL41 showed in combined EFCJ group the increase of CD3+ IEL and LPL on 4 dpi. Cecal CD4+ LPL elevated on day 4 and IEL on day 7 pi. CD8+ showed only IEL improvement on 7 dpi. On the other hand, while IgA+ IEL not overnumbered CJ group, LPL raised on 4 and 7 dpi. *Lactobacillus fermentum* CCM 7514 presented in combined LBCc group later increase of IEL CD3+ and CD4+ (7 dpi), but earlier improvement of IEL and LPL CD8+ together with IgA+ (4 dpi). Both probiotic strains showed beneficial effect to campylobacters presented mainly by IgA+ increase, showing the contribution of humoral immune response. Acknowledgements: Thank you to the Grant Agency for Science of Slovak Republic VEGA 1/0562/16, 1/0112/18 and Slovak Research and Developmental Agency APVV-15-0165.

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UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI SCIENZE VETERINARIE PER LA SALUTE, LA PRODUZIONE ANIMALE E LA SICUREZZA ALIMENTARE

Praeoperative antibiotics in equine uncomplicated clean orthopaedic surgery - A randomised controlled study.

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Abstract

Several studies report that there is no association between septic arthritis after arthroscopy or the development of post-operative complications and perioperative antimicrobial prophylaxis and low rates for septic arthritis after arthroscopy without perioperative antimicrobial prophylaxis. The following prospective randomised study examined the development of post-surgical complications in these types of surgery with /without perioperative antimicrobial prophylaxis. 75 horses underwent elective orthopaedic surgery and were randomly assigned to either a treated or a control group. As prae-operative antimicrobial prophylaxis, treated horses received antibiotics as a single-shot (6.6 mg/kg gentamicin, 10 mg/kg amoxicillin) 30 to 45 minutes prior to surgery. Controls received neither antibiotic nor placebo. The horses stayed at the clinic for at least five days after surgery. The rectal body temperature was measured in the mornings, at day 0-3 also in the evenings. Bandages were changed at day 1, 3 and 5 and score points for exudation (0, 1 or 3), swelling (0-3), skin temperature (0-3), and dehiscence (0-3) assigned. Differences between treated and control horses were detected with a two way ANOVA with repeated measures. Fisher's exact test and the Wilcoxon-Mann-Whitney-test were used as post-hoc tests. 59/75 horses (78.7%) were considered for statistical analysis. Reasons for drop out were unrelated to group assignment. Treated horses had a significantly higher total score than control patients for swelling and skin temperature. For exudation and dehiscence, there was no significant difference. Also, there were no significant differences between the groups in body temperature. At day 8 and 9 respectively, one control and one treated (dropped out, additional gentamicin) horse developed septic arthritis. As wound infections after clean elective orthopaedic surgery are rare, patients should be monitored carefully for at least 20 days especially after arthroscopy. Nonetheless, the single-shot praeoperative administration of antibiotics failed to reduce wound scores and eliminate postoperative fever in this study. A routine, praeoperative application of antimicrobials in these type of surgeries seems unnecessary. The administration of pre-/post-operative antibiotics in equine clean elective surgery requires an individual risk/benefit assessment including surgical and postoperative findings.

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Vespa

Friend or foe? vertical integration and spread of antimicrobial resistant organisms.

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Abstract

This study evaluated the usefulness of antimicrobial phenotyping of Salmonella enterica subsp. enterica serovar typhimurium for investigating transmission between locations within a vertically integrated poultry operation. A longitudinal study was conducted within an "antimicrobial free" vertically integrated chicken meat operation between Jan 2013 - July 2014. Transmission pathways were identified within the enterprise using social network analysis (SNA). Sixty percent of all locations within the enterprise were longitudinally sampled to detect Salmonella spp. Salmonella typhimurium isolates were screened for antimicrobial susceptibility with a panel of 11 antimicrobials using the Calibrated Dichotomous Sensitivity (CDS) and minimum inhibitory concentrations (MIC) were determined using Sensititre. A selection of 411 isolates were whole genome sequenced (WGS) and the correlation of antimicrobial phenotypes between sampling locations was investigated using diversity analysis. A total of 4,219 environmental samples were collected for testing. Sixty three percent of positive samples were identified as Salmonella typhimurium. Two clonal lineages of S. typhimurium were identified in this population. The phenotypic test identified 16.5% of isolates susceptible to all antimicrobials tested and three resistant phenotypes; sulphafurazole (68.5%), streptomycin (56.5%), and ampicillin (10.1%). No fluoroquinolone, cephalosporin or ESBL producing phenotypes were identified. MIC testing confirmed 10%, 8% and 2% of these phenotypes respectively as above clinical breakpoint values. No transmissible genes conferring resistance to sulphonamides or streptomycin were identified in this population. Four different 4 TEM ß-lactamase genes were the only transmissible resistance genes identified in 3.4% of the sequenced isolates. Non-susceptible isolates were not clustered by location and the dissemination pathways identified using SNA and confirmed by whole genome sequencing were not supported by AMR phenotyping. A clonal population of Salmonella typhimurium was detected within the vertically integrated enterprise. The transmission pathways identified by SNA and confirmed by sequencing were not supported by susceptibility phenotyping. Social network analysis revealed that the introduction of a single bacterial isolate into a vertically integrated operation would result in rapid dissemination between locations even when the reproductive ratio within the system was less then 1 ($R_0<1$). These results were supported by whole genome sequencing of the isolates. Susceptibility testing has been used as another method of identifying isolates to a putative source. These results demonstrate that this assumption should be used with caution and that more work is required to understand the transmission epidemiology of important zoonotic pathogens and antimicrobial resistance.

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Is the antibiotic-free labelling helping the fight against antibiotic resistance?

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Abstract

Responsible use of antibiotics is a growing need that can no longer be ignored. Several countries have been setting up targets for antibiotic use. This growing awareness reached consumers, leading companies to respond with a myriad of different claims, which the vast majority is turning into an antibiotic-free message. In this paper we analyse whether this movement from the industry is leading to lower use of antibiotics and to a movement away from antibiotic misuse. The "antibiotic-free" label indicates that the product has come from animals that were not subject to antibiotic treatments at any point in their life. However, there is a range of interpretations which may apply to just part of an animal's life cycle, a proportion of the supply chain, or be limited only to therapeutic use. For instance, in some cases the antibiotic-free label might be achieved by: 1) Increasing the use of other treatments such as ionophores (or other coccidiostats), pro- and pre-biotics and vaccinations; 2) improving traceability, being able to trace animals that did not need antibiotics and direct them to a parallel "antibiotic-free" supply chain; 3) selling products with claims such as "antibiotic free in the last X months of life" thus excluding early stages of life where the wider use of antibiotics is usually done. These solutions do not really address the fundamental problems that lead to antibiotic over use and misuse: bad management practices and poor animal welfare. Good management practices such as biosecurity and good feeding and watering influence the health status of any farmed animal. Also, a change on the way animals are raised and bred needs to happen, with systems being designed according to the animal needs. Providing them with a good quality of life, including lower stocking densities and provision of environmental enrichment, improves their welfare, as well as their capacity to respond to infectious challenges. Good management and good animal welfare should always underpin a plan for responsible use of antibiotics. Simply making a statement on a label will not help address the irresponsible use of antibiotics in farm animals if systems are not redesigned first.

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Linking environmental contaminant PCB on tetracycline resistance in Enterococcus faecalis.

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Abstract

Among environmental contaminants polychlorinated biphenyls (PCBs) are still a concern due to their persistence in environment. As an emerging contaminant, they are directly linked to climate change and few studies are available for their relation between microbiota including bacteria. Antibiotic resistance (AMR) as an increasing global issue, is still to be debated for its relation with pollutants. This study was aimed to determine the effects of dioxin-like PCB (PCB118) on tetracycline (Tet) resistance in Enterococcus faecalis ATCC 29212. Tet resistant strains were obtained by inducing tetracycline at 2-125 ppm; where the strains were exposed to Tet at increasing concentrations for cumulative 14 days to be transferred from nutrient agar to nutrient broth every day. PCB118 at 100 ppt in isooctane were added to tetracycline resistant strains. 6 groups were studied where bacterial strains in cultured broth of Group 1 received PCB and Tet, Group 2 received isooctane and Tet, Group 3 only Tet, Group 4 only PCB, Group 5 only isooctane and Group 6 broth only as a bacterial control. Antimicrobial susceptibility were determined by Kirby-Bauer disc diffusion and standard broth microdilution method for AMR as defined by CLSI guidelines. All experiments were run in triplicates. Zone diameters were found to be lower (27%, $p<0.05$) and MIC values were higher (29%, $p>0.05$) in Group 1 compared to Group 3. Indicating that PCB treated group were more resistant compared to Tet only treated group. MIC results of Group 2 and 5, which both received isooctan, indicate that isooctan only group were more susceptible; where cytotoxic effects of the organic solvent were thought to have influenced in vitro. This is the first study to directly link antibiotic resistance on PCB presence in E. faecalis in vitro. The preliminary experiment indicates that environmental contaminant, PCB118, induce positive effects on tetracycline resistant E. faecalis strains, where resistance were found to be increased. E. faecalis, as an environmental pathogen, where the resistant genes survive though sewage treatment, increase of the viability with respect to PCB polluted areas increase risk for human health. Further in vivo studies revealing the mechanisms of resistance-PCB relation are expected to confirm.

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Pharmacokinetic/pharmacodynamic analysis of metronidazole and imipenem used to treat *Bacteroides spp*. infection of the pleural cavity in horses.

Taisuke Kuroda[1,*], Shun-ichi Nagata[2], Yuta Kinoshita[1], Hidekazu Niwa[1], Norihisa Tamura[1], Kentaro Fukuda[1], Hiroshi Mita[1], Yoshinori Kasashima[1]

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Abstract

Obligate anaerobic bacteria, including *Bacteroides spp*., have been known to be highly pathogenic in equine pleuropneumonia. Metronidazole and carbapenems are effective to control obligate anaerobic bacterial infection in humans. To determine an antimicrobial therapy for the infection of the pleural cavity of horse, pharmacokinetics (PK)/pharmacodynamics (PD) analysis was done based on pleural fluid concentrations of metronidazole and imipenem and their minimum inhibitory concentrations (MICs) against *Bacteroides spp*. isolated from horses with pleuropneumonia. Four horses were administered 15 mg/kg metronidazole orally and 10 mg/kg imipenem intravenously in a random crossover study design with a 4-week washout period between administrations. Pleural fluid was collected at 1, 3, and 8 h, and blood was collected at 0, 0.08, 0.17, 0.33, 0.5, 0.67, 0.83, 1, 1.25, 1.5, 2, 3, 4, 6, 8, 12 and 24h after drug administration. Drug concentrations were measured using high performance liquid chromatography. MICs of both antimicrobials against 22 *Bacteroides spp*. strains isolated from horses with pleuropneumonia were evaluated using gradient strip tests. The highest pleural concentrations of metronidazole and imipenem were 12.7 ± 3.3 μg/ml and 12.1 ± 0.9 μg/ml at 1 h after administration, respectively. The pleural concentrations of both antimicrobials were similar to the plasma concentrations at all time points, indicating that they were efficiently penetrated to the pleural fluid. The MIC90 values of metronidazole and imipenem were found to be 4 μg/ml and 0.5 μg/ml, respectively. It was indicated that the target PK/PD parameter for metronidazole was an AUC0–24 h/MIC ratio>70. PK simulation of metronidazole at a dose of 15 mg/kg at an 8-h interval administration indicated that the average AUC0-24 h was 339.6 μg.h/ml, and the AUC0–24h/MIC90 was found to be 84.9. In carbapenems, it was indicated that the target PK/PD parameter was time above MIC (TAM) > 50 % per day. The TAM of imipenem at a dose of 10 mg/kg at 8-h interval administration was 70.8 % per day. Thus, 15 mg/kg 8-hour interval metronidazole oral administration and 10 mg/kg 8-hour interval imipenem intravenous administration are expected to control *Bacteroides spp*. infection of the pleural cavity in horses.

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Intramuscular administration of sodium benzylpenicillin with addition of lidocaine and adrenaline in horses.

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Abstract

Benzylpenicillin is the most commonly used antibiotic in veterinary medicine in Sweden. Only procaine benzylpenicillin (pc-prok) is approved for intramuscular administration in horses. The procaine is associated with the sometimes severe adverse reaction "penicillin shock" (1) and intramuscular treatment with sodium benzylpenicillin are shown to be painful (2). The aim of the present study is to investigate if sodium benzylpenicillin with addition of lidocaine with adrenaline (pc-lido) could be used as an alternative. The study was randomized, blinded and a two treatment crossover design. Eight horses were given sodium benzylpenicillin (6.25 mg/kg) with addition of lidocaine (0.1 mg/kg) with adrenaline (0.05 µg/kg) twice daily or pc-prok (as control, 21 mg/kg, once daily) intramuscularly for four days. Blood samples were analysed for plasma benzylpenicillin. Using the variation from the studied population, the plasma concentrations of benzylpenicillin was simulated (Monte Carlo simulation) in 200 horses, and compared to MIC (Minimum Inhibitory Concentration) for common pathogens in the horse. The behaviour of the horses was recorded before and after each morning injection. The mean (±SD) half-life of benzylpenicillin after intramuscular administration of pc-lido was 2.6 ± 1.0 h. The fT > MIC for Staphylococcus aureus (MIC = 0.125 µg/mL) was 80 % ± 23. The Monte Carlo simulations show that in all simulated horses treated with pc-lido, the fT > MIC (MIC = 0.125 µg/mL) exceeds 50 % of the dosing interval. A fT > MIC of 30-50 % or more is considered appropriate for antimicrobial treatment with penicillin (3, 4, 5). There was no significant difference in the behaviour of the horses receiving pc-lido compared to control. In conclusion, pc-lido twice daily (6.25 mg/kg) appears to be adequate antimicrobial therapy to treat many common infections in the horse and using pc-lido would eliminate the risk of adverse procaine reactions.

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Pharmacokinetics of antimicrobial agents in turtles.

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Abstract

The North Carolina State University Veterinary Hospital Turtle Rescue Team provides medical care to injured wild turtles presented for treatment. These turtles are the victims of vehicular accidents, animal attacks, and fish hook trauma. During hospitalization, the turtles are highly susceptible to fungal and bacterial infection, which significantly delays their recovery and return to the wild. Our laboratory has developed unique antimicrobial agent protocols for treating these patients with the use of population pharmacokinetic studies and pharmacokinetic-pharmacodynamic (PK-PD) principles. Because of their size, blood volume, and the stress of frequent blood sampling, we have applied sparse-sampling and population pharmacokinetic methods. Our studies employ nonlinear mixed-effects modeling (NLME) and the Phoenix software system (Phoenix® NLME™ version 7.0, Certara Inc., St. Louis, Missouri) for analysis. The NLME approach estimates the typical value fixed effect) and between-subject variability, BSV (random effect) for the population. This approach also can examine covariates in the population that account for BSV. Our studies show substantially prolonged elimination rates compared to mammals for antimicrobials eliminated by renal clearance mechanisms. Because of these observations, we have utilized antibiotics such as fluoroquinolones and cephalosporins because they can be administered infrequently, and are active against pathogens that cause infections in these turtles. Our most recent studies used NLME modeling and sparse sampling of 3-4 samples per animals to determine the pharmacokinetics of ceftazidime and enrofloxacin. For ceftazidime (24 turtles), the half-life was 35 hours, VD 0.26 L/kg, and clearance 0.01 L/kg/hr. For enrofloxacin (71 turtles), the half-life was 76 hours, VD 0.81 L/kg, and clearance 0.02 L/kg/hr. The long half-life of ceftazidime allows for a dose of 20 mg/kg every 5 days to maintain drug concentration above the MIC of susceptible bacteria. Our studies with enrofloxacin showed that ciprofloxacin was a minor metabolite (less than 8% of total), but because of the long half-life and high AUC, enrofloxacin can be administered 10 mg/kg once every 3 days (or less frequently) and produce sufficient AUC/MIC concentrations for susceptible bacteria.

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Development and evaluation of a single intramuscular, controlled-release florfenicol formulation for use in pigs.

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Abstract

Swine respiratory disease (SRD) is amongst the most serious disease problems in modern swine production and often requires antimicrobial therapy. In most cases, antimicrobial administration is given to groups of pigs via feed or water and by using this method there are animals that are not ill but receive treatment. This unnecessary administration increases the chance of resistant bacteria formation. Administration by injection is less common and often requires multiple injections which is time consuming and involves intensive use of labor. There are some new sustained release formulations of cephalosporins (3rd & 4th generation), fluoroquinolones and macrolides that may only require a single injection for a full course of therapy; however, these antimicrobial types are critically important for human use and their veterinary use is being increasingly limited because of resistance concerns. Florfenicol (FFC) is not in human use and is highly effective for treatment of infections in SRD. There are conventional FFC injectable products available on the market; however, those require at least 2 injections that again involve labor and time. Another issue to be mentioned is the stress that these animals go through each time they are repeatedly injected. Therefore, there are great advantages to using a single dose of FFC. The aim of this study was to develop and evaluate an injectable, bio-degradable, controlled release intramuscular (IM) FFC formulation to achieve single dose therapy of pigs. Single dose injectable administration of antimicrobials provides the advantage of rapid and targeted, individual animal treatment without the need to re-handle each pig for subsequent injections. The concept is based on an injectable FFC, which is incorporated in a reverse thermal gelation system using poloxamer polymers. The suspension is liquid at ambient temperatures and is easily injected, after which it turns into a soft gel at body temperature. The drug is released by diffusion from the gel and/or by erosion of the matrix following dissolution. We have compared the plasma concentrations of FFC in pigs after IM injection of our prototype formulation with that after two separate doses of a commercial product. While commercial FFC plasma concentrations decreased below MIC90 after less than 12 hours, our prototype maintained plasma levels above the desired concentration for more than 4 days. These preliminary results are very promising and we plan to complete additional studies in order to strengthen the concept.

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UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI SCIENZE VETERINARIE PER LA SALUTE, LA PRODUZIONE ANIMALE E LA SICUREZZA ALIMENTARE

The relationship between antimicrobial use and herd level mortality as a welfare indicator in veal calves.

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Abstract

The veal calf industry urgently needs to reduce antimicrobial use (AMU). A huge challenge, since this industry holds major risk factors for disease (e.g. young age, commingling, transport stress). As a result, fear is that a rapid decrease would increase mortality and therefore hamper animal welfare. This study explored the relationship between AMU and mortality in two major veal calf companies (integrations). Total AMU and different classes, including critically import antibiotics, were added to the model. The secondary objective was to determine risk factors for mortality. A retrospective cohort study was performed on electronically collected antimicrobial consumption and mortality data from the largest Belgian veal practice in the period 2014-2016. A mixed linear model was built to identify factors associated with mortality. The data consisted of 76 production cycles from 29 different farms managed by two integrations (1 and 2), covering 45.001 dairy veal calves. Average AMU was 30.1 defined daily doses for animals per year (DDDvet/year, standard deviation (±) 10.4), and was significantly higher in integration 2 (35.9 DDDvet/year ± 9.3) compared to 1 (22.4 ± 5.7). In contrast, integration 2 had significantly lower mortality than integration 1 (2.3% ± 1.4 vs 4.1% ± 1.4). Analysis showed a positive association between AMU and mortality in production cycles of integration 1 and no association at all in integration 2. In the final multivariable model, the mortality risk increased with an increasing herd size and with the use of 3th/4th generation cephalosporins. Whereas mortality risk decreased with an increased use of long-acting macrolides. This study shows that at the current levels of total and critically important AMU in Belgian dairy veal calves, a decreasing use was not associated with increased mortality. In contrast, in the veal company with the lowest AMU, a positive association with mortality was observed. These data suggest that no welfare issue was induced so far and that selection of appropriate antimicrobial therapy by the veterinarian as well as identification of the veal company as an influencer of AMU and mortality will be crucial to further rationally reduce AMU in veal calves.

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Vespa

Knowledge and attitudes on antibiotic use and antimicrobial resistance among veterinary students.

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Abstract

Antimicrobial resistance (AMR) poses a rapidly increasing worldwide problem and it is to a certain extent related to lack of knowledge among healthcare professionals. Training of all healthcare professionals is highlighted in the WHO's Global Action Plan on AMR to combat this emerging public health problem. Irrational use of antibiotics is directly associated with AMR and according to the report of the National Reference Laboratory for Antimicrobial Monitoring and Surveillance, Serbia belongs to the up of European countries with the highest rates of AMR. This study aimed to evaluate the knowledge and attitudes of the veterinary students towards antibiotic use and AMR. The study was conducted at the Department of Veterinary Medicine, University of Novi Sad, among 105 veterinary students (3rd, 4th and 5th year of study). They attended Pharmacology course and agreed to complete the anonymous questionnaire. The average age of students was 22.9 years and 49.5% of the respondents were females. Around 45.7% of the students used antibiotics to treat common cold and 18% of them used antibiotics to treat fever. Although 97.1% of students know that antibiotic treatment should be started following a visit to a medical doctor and receiving a prescription, only 63.8% obtained antibiotics with prescription from a medical doctor during the last infection. Roughly 32.4% of the students thought that treatment with antibiotics should be started on the basis of pharmacist's advice and around a third of the respondents practiced self-medication with antibiotics when they were ill. This study suggests that veterinary students are aware of the importance of AMR, but many still have certain misconceptions. Therefore, further educational interventions should be focused on improvement of their perceptions and attitudes towards AMR and on applying behavioral insights methodology in regard to antibiotic use.

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Application of reverse line blot hybridization assay for the detection of various tick-borne pathogens in Pakistan.

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Abstract

Tick-borne diseases (TBDs) have a damaging impact not only on the animal health, but also poses serious risks to human health resulting in heavy burden on national economy particularly in developing countries like Pakistan where livestock has a significant share in GDP. Although climatic and ecological conditions in Pakistan may favour the transmission of tick-borne pathogens, to date, there is no study in Pakistan where the authors efficiently utilized Reverse Line Blot (RLB) hybridization assay for the detection of various tick-borne pathogens. This technique can detect multiple pathogens in a single run, therefore it forms the basis for early detection and control of TBDs. A total of 3,807 ticks were collected and pooled based on their locality, host and species. These pools were screened for several pathogens using PCR in combination with RLB hybridization assay. The identified tick-borne pathogens not only belonged to endemic species in Pakistan, such as Anaplasma ovis (1.5%), Babesia bigemina (0.7%) and Babesia bovis (0.2%), but also several tick-borne pathogens were found for the first time in Pakistan. These included Ehrlichia minasensis (3.2%), an Anaplasma platys-like organism (1.2%) and Rickettsia spp. (1.2%), as well as two previously uncharacterized species: Ehrlichia sp. Multan and Anaplasma sp. (BL099-6). The pathogenicity of these novel species remains to be examined. This study highlights the importance of RLB assay in early detection of wide range of pathogens, including novel agents, using minimal resources and short turnaround time which otherwise would have exhausted more resources. The study also reveals that a wide range of tick-borne pathogen species, including many zoonotic pathogens, is prevalent in Punjab, Pakistan.

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UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI SCIENZE VETERINARIE PER LA SALUTE, LA PRODUZIONE ANIMALE E LA SICUREZZA ALIMENTARE

Gain edge from bridging animal and human ESBL-producing *E. coli* surveillance.

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Abstract

Human and animal exchange of antimicrobial resistance (AMR) genes occurs by direct contact, food borne or from environment, subsequent spread requires specific risk factors in the receiving population, mostly exposure to antimicrobial consumption. Risk mitigation would benefit of rapid detection of new multidrug-resistant (MDR) organisms. We explored the possibility of using data from antimicrobial susceptibility testing (AST) in humans to support target AMR surveillance in humans and animals. Between 2016-2018 we run a cross sectional study to verify shared antimicrobial phenotypes and molecular features in ESBL-producing E. coli (*E.coli*-ESBL) from humans and animals. Overall, 953 *E.coli*-ESBL isolates from humans (53%) and food-producing animals (47%) were collected in six Italian Regions. Human clinical isolates were from urine and blood samples; animal isolates were detected in intestine of diseased bovines, swine and poultry. *E.coli*-ESBL from both sources were tested for *bla*CTX-M, *bla*SHV and *bla*CMY ESBL, *mcr*-1 and *mcr*-2 and genotyped by phylogenetic typing; a subgroup of isolates was subjected to MLST. Human public laboratories of one of the above six Italian Regions used harmonized management system to collect AST data from clinical cases from 15 hospitals and their reference areas. Among data on 29,000 presumptive ESBL-*E.coli* isolates collected in 2014-2015, an anomaly in the distribution of colistin susceptibility was detected in three hospitals. These clusters of colistin-resistant isolates have not been further investigated for detection of colistin resistance-associated genes but *mcr*-1 was found to circulate in the same Region during the cross-sectional study (2016-2018). Overall, results from the cross sectional study supported current knowledge of human *E.coli*-ESBL mostly (393, 77.4%) belonging to B2 phylogenetic group, while fewer (19, 4.3%) animal isolates were classified in this group. Among B2 human isolates, 321 (87.5%) belonged to the successfully pandemic ST131 clone and four of them carried both *mcr*-1 and *bla*CTX-1 group. Among B2 animal isolates, three belonged to ST131, and one, from poultry, carried both *mcr*-1 and *bla*SHV-12, but not *bla*CTX-M. The remaining 36 animal isolates *mcr*-1-carrying belonged to clones other than ST131. AMR surveillance may gain edge from medical-veterinary integration.

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High diversity and spread of mcr transferable genes encoding colistin resistance among multidrug-resistant isolates from primary productions in Italy.

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Abstract

In 2015, colistin resistance mediated by mcr-1 gene located on a transferable plasmid was first reported in Escherichia coli from animals, food and patients from China. Subsequently, almost all European countries, detected the presence of mcr. At present (July 2018), 7 different mcr genes and variants has been described. Colistin is a last resort antimicrobial for the treatment of infections by carbapenem-resistant Enterobacteriaceae in humans, and a Highest Priority Critically Important Antimicrobials for human medicine (WHO). E. coli and Salmonella isolated in the EU harmonised antimicrobial resistance monitoring in 2014 and 2015 from food-producing animals, testing colistin resistant and almost invariably also multidrug-resistant, were analysed by multiplex PCR for the detection of mcr-1 to mcr-5 genes and whole genome sequenced (WGS). Colistin resistance was mainly observed in the ESBL/AmpC-producing E. coli population, and was present in 25.9%, 5.3%, 6.5%, and 3.9% of such isolates in turkeys, broilers, pigs, and bovines, respectively. Most isolates (141/161, 87.5%) harbored genes of the mcr-1 group. mcr-1 was also detected in a small proportion of Salmonella isolates (3/146, 2.0%) in turkeys. Bioinformatics analysis on WGS isolates revealed a high diversity of mcr variants. In turkeys we detected mcr-1.1, mcr-1.2 and mcr-1.13, in pigs, mcr-1.1, mcr-1.13 and mcr-4.2, and in bovines, mcr-1.1, mcr-3.2 and mcr-4.3. All the mcr genes were located in a transferable plasmid, except for mcr-1.13 (inserted in a mobile genetic element in the chromosome). The mcr variability in the E. coli and Salmonella from the Italian primary productions could constitute an advantage for these microbial populations exposed to continuous antimicrobial selective pressure. Quick and thorough action should be taken by Competent Authorities and by the farming industry to drastically reduce the use of colistin in animals, following the recommendations of the European Medicines Agency (at least to a ≤5 mg/PCU). We also strongly recommend reducing the overall use of all other classes of antibiotics at primary production level, in order to mitigate the effects of the complex mechanisms behind co-selection and multidrug resistance towards Critically Important Antimicrobials, in a "Consumer Protection" and a "One Health" perspective.

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Optimizing identification of mycoplasma bovis by MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry).

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Abstract

Mycoplasma bovis is an important bovine pathogen causing primarily pneumonia, otitis and arthritis in calves, and pneumonia and mastitis in adult cattle. Mixed infections of *M. bovis* with other less or non-pathogenic Mycoplasma species in clinical samples are possible. While cultivation is inexpensive and allows strain typing after isolation, definite identification requires expensive and time-consuming techniques. Nevertheless, early detection of *M. bovis* is highly important in order to rationalize antimicrobial use. MALDI-TOF MS is a fast technique for identifying pathogens in routine diagnostics. However, quality and reproducibility of spectra can be influenced. Therefore, the objective of this study was to explore growth conditions and incubation time to optimize *M. bovis* identification. A single colony of three different M. bovis strains was inoculated several times in 25 mL of four different broths (B1-4). Basic broth (B1) consisted of pleuropneumonia-like organism broth, enriched with 25% horse serum and 0.07% yeast extract. B2, B3 and B4 were additionally supplemented with pyruvate (0.5%), polysorbate 80 (1.0%) and ampicillin (0.01%), respectively. Protein extraction was performed at 0, 24, 48, 72, 96 and 120 hours after incubation at 37°C in a 5% CO2-enriched atmosphere. Spotted supernatant was processed with an Autoflex III smartbeam MALDI-TOF MS (Bruker Daltonik). Identification, interpreted as reliable with a score ≥1.7, was best after 48 and 72h in B1 (93-100%) and B2 (100%). At 0h no identification was possible and B3 failed identification at all times. After 24h, 63-85% (B1, 2, 4) of the M. bovis isolates were identified, with significant higher identification rates in B2. After 72h identification rates reduced drastically in B1 and B3. Identification scores with B2 remained similar until the end of the experiment. In conclusion, starting from one colony, reliable and cheap *M. bovis* identification is possible after 48h with MALDI-TOF MS. Use of polysorbate 80 is discouraged, however adding ampicillin to the medium may be useful to avoid contamination. Adding pyruvate to the medium ensured fast reliable identification after 24 hours of incubation and persisted up to five days after inoculation. This information can be useful to improve MALDI-TOF MS assisted diagnosis of *M. bovis* in peripheral laboratories.

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An innovative approach for analysing and evaluating poultry farms odour related to animal health and welfare.

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Abstract

Volatile organic compounds (VOCs) produced by pathogens, host-pathogen interactions and biochemical pathways were explored in several study studies as biomarkers for their capacity of diagnosing pathologies in livestock and in humans. VOCs are present everywhere such as in blood, breath, faeces, sweat, skin, urine and vaginal fluids and their qualitative and quantitative composition is influenced by pathophysiological responses to infections, toxins or endogenous metabolic pathway perturbations. In poultry, VOCs analysis has been explored to evaluate air quality in sheds, but they have never been monitored to determine if birds were affected by enteric pathologies. These enteric disorders represent one of the most important groups of diseases they affect poultry and cause illness, mortality and economic losses. For this reason, monitoring the health status of broilers and an early detection of any health problem is of great importance in intensive farming, especially nowadays that antibiotics are banned. Nowadays, the antibiotic as a prophylaxis in intensive farming system, is still in use and this management practice may cause spreading of drugs in the environment, contributing to the phenomenon of antibiotic resistance. The prompt reaction to any change in health, welfare and productive status is the key for the reduction in drugs usage and for the improvement of animal wellbeing. Due to the high priority of this issue, it is of great importance the early detection of any health problem in intensive farming. Precision Livestock Farming (PLF), through the combination of cheap technologies and specific algorithms, can provide valuable information for farmers starting from the huge amount of data collected in real time at farm level. This study was aimed to the application of a PLF diagnostic tool, able to detect the variation of volatile organic compounds, to promptly recognize enteric problems in intensive farming, supporting veterinarians for selecting specific treatments in case of disease.

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MCR-1 and B-Lactamases in *Escherichia coli* resistant to extended-spectrum cephalosporins isolates from Italian dogs.

Fabrizio Agnoletti[1], Romina Brunetta[1,*], Ilenia Drigo[1], Elena Tonon[1], Silvia Deotto[1], Laura D'Este[2], Antonio Barberio[1], Monia Cocchi[1], Gabriella Conedera[1], Michela Corrò1, Deborah Dellamaria[1], Karin Trevisiol[1], Elena Mazzolini[3]

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Abstract

Close cohabitation between pet and their owners allows transmission of Escherichia coli from dogs to humans and vice versa. E. coli carrying extended-spectrum β-lactamases (ESBL-E.coli) pose a serious risk of therapy failure in humans and strict surveillance of any ESBL-E.coli reservoir is of importance for risk mitigation. Aim of this preliminary work was to describe the β-lactamases involved in ESBL-E.coli isolates from dogs and to verify the occurrence of mobile colistin resistance genes mcr-1 and mcr-2. In 2016–2017, for 16 months, 303 dogs were selected by systematic sampling among dog carcasses or dog faecal samples received for necropsy or parasitology screening at the IZSVE laboratories (north-east Italy). Presumptive ESBL-E.coli were isolated from faecal samples or intestinal content by selective media added with cefotaxime. One presumptive ESBL-E.coli isolate per dog was further investigated by antimicrobial susceptibility testing and molecular methods to confirm β-lactamase resistance and detect carbapenemases. Among selected dogs 15% (45/303) harbored ESBL-E.coli, while no carbapenem-resistant isolates were found. Isolates were screened for AmpC and ESBL by conventional PCR, and further in-depth identification of the bla genes was performed by sequencing. Overall 16/45 isolates harbored blaCTX-M1 group genes, 15/45 of which blaCTX-M15, 6/45 isolates harbored blaCMY-2, 4/45 blaTEM-1 and 2/45 blaSHV-12. In the remaining 17/45 isolates various associations of β-lactamases were found: the blaCTX-M15 and blaTEM-1 association was detected in 9/45 isolates, the blaTEM-1 and blaCMY-2 association in 5/45 isolates, the blaTEM-1 and blaSHV-12 association in 2/45 and the blaSHV-12 and blaCTX-M1 group was detected in 1/45 isolate. Four/45 ESBL-E.coli, 2 blaCTX-M15, two blaTEM-1 and blaCMY-2 and one blaCMY-2, belonged to B2 phylogenetic group, the most represented among human ESBL-E.coli isolates. Three isolates harbored mcr-1, two were associated to blaCTX-M15, belonging to phylogroups B1 and D, and one to blaTEM-1 belonging to phylogroup E. Our results show moderate variability of bla involved in ESBL-E.coli from dogs and confirm that in this pet animal, as in humans, TEM-type and SHV-type are outnumbered by the CTX-M ESBL class. The occurrence of mcr-1 in three ESBL-E.coli isolates is of concern, yet the phylogenetic group points to the animal reservoir origin.

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MRSA still low prevalent in North-Eastern Italian dairy herds.

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Abstract

Methicillin-resistant Staphylococcus aureus (MRSA) is seldom reported in dairy cattle yet when it enters a herd it spreads among animals, causing sub-clinical intramammary infection and providing a long-term reservoir of exposure to farmworkers. Clinical samples from dairy herds can be used for scanning surveillance based on at-risk sampling. To estimate MRSA prevalence in dairy herds reporting mastitis (all mastitis) in north-east Italy we set up an observational study from March 2016 to October 2017 (19 months) by selecting 977 dairy herds among those submitting milk samples to the IZSVe laboratories (north-east Italy) for screening of mastitis agents. The size of herd sample was intended to provide a prevalence estimate supposing the highest uncertainty on true prevalence, 95% confidence and 7% precision; to avoid over-clustering the sample size was 3 times multiplied and herds were sampled only once over the study period. The herd sample enrolled in the study was distributed over the reference population of the study area according to single provinces and representing the average of the proportion of herds and dairy cows of the province. Milk from clinical samples was pooled and inoculated in cefoxitin and aztreonam selective medium. S. aureus colonies tested mecA-positive and resistant to oxacillin and/or cefoxitin were classified as MRSA, mecA-negative were further screened for mecC. MRSA was detected in pooled milk from clinical samples of three herds out of 977 selected. In two herds MRSA was characterized by spa typing and multilocus sequence typing (MLST), and in both MRSA ST398 spa type t034 was detected. Two dairy herds were further investigated by thorough within herd sampling and MRSA detected in single cows, environment and fomites. Given the low (0.3%) prevalence of MRSA contaminated dairy herds the screening of MRSA among mastitis agents should be performed by using high sensitive laboratory methods.

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UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI SCIENZE VETERINARIE PER LA SALUTE, LA PRODUZIONE ANIMALE E LA SICUREZZA ALIMENTARE

Evaluation of microbial community composition of dairy cows manure and soil before and after its fertilization.

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Abstract

Microbiota is the entire collection of microorganisms in a specific environment and microbiome is all the genetic material within it. This is a complex system influenced by variations in its ecosystem itself, i.e. soil and gut. Bovine manure is commonly used for the fertilization of agricultural soils and it comprises the intestinal microbiota influenced both by feed and possible residues of drugs used for therapy. The excessive use of antibiotics in animal husbandry and subsequent land application of animal wastes may introduce massive quantities of antimicrobial drugs (AMD) and resistant bacteria into the soil environment. A research project has been granted by the Italian Ministry of School Education, University and Research (PRIN 2015KA3XFH) to evaluate the role of intensive animal farming as potential source of environmental antimicrobial contamination and resistance. To this aim samples of manure and soil (before and 30 days after fertilization) were collected from intensive dairy cow farms located in Veneto and Lombardia regions, where intensive animal farming is widespread. All samples were screened for detecting and quantifying the commonly used AMDs (i.e. beta-lactams, fluoroquinolones, polymyxins and macrolides) by HPLC-MS methods. The microbiome of the samples was determined by employing a culture independent approach based on metagenomics and NGS-sequencing. All samples were negative for AMDs selected. The manure microbiome was homogenous among the different farms and regions, but very different from the soil one. In manure a higher presence of *Porphyromonadaceae, Lachnospiraceae, Clostridiaceae, Pseudomonadaceae* and *Ruminococcaceae* families was observed, whereas in soil the *Chitinophagaceae* family was the most represented. After fertilization, the microbiome composition was not modified in soil, however the absence of drugs concentration in manure may have had a role in this process. The research project will be completed by assessing the prevalence and the diversity of antimicrobial resistance genes to multiple antimicrobial classes by quantitative real time PCR. This is one of the first studies that evaluates the modifications in the microbial communities of manure and soil before and after fertilization. Thanks to the metagenomics analysis, this research project may elucidate the role of intensive animal farming in the diffusion of antimicrobial resistance in the environment.

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INCX4 plasmid harbours mcr-1.1-mediating colistin resistance in the emerging pESI-positive, ESBL-producing, multidrug resistant Salmonella infantis clone isolated in Italy from broilers and broiler meat (2016-2017).

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Abstract

Salmonella enterica serovar Infantis represents the most frequent serovar detected in broilers and broiler meat and one of the top five serovars involved in human infections in Europe. The increasing incidence of S. *infantis* infections may be complicated by the spread of multidrug-resistant (MDR) strains, such as the recent spread in Europe of MDR, Extended Spectrum Beta-Lactamases (ESBL)-producing S. *infantis* harbouring a conjugative pESI-like megaplasmid in broilers, broiler meat and humans. In this study we report for the first time the isolation and in-depth characterization by whole genome sequencing (WGS) of four, *mcr-1*-positive, MDR S. *infantis* isolated from broilers and broiler meat samples in the frame of the Italian antimicrobial resistance monitoring (2016-2017). Phylogenetic relationships with previously characterized Italian S. Infantis, belonging to the pESI-like positive, (ESBL)-producing clone, were also assessed. All isolates presented the same *gyr*A point mutation associated with fluoroquinolones resistance and harboured: a. a pESI-like plasmid containing genes coding for different toxin-antitoxin systems and specific markers associated with virulence, enhanced colonization capability and enhanced fitness, previously described in pESI-like-positive S. *infantis* in Italy; b. an incX4 plasmid harbouring *mcr-1.1* and the genes coding for the HicAB toxin-antitoxin complex. Additionally, two isolates were also ESBL-producers (CTX-M-1 type) and within the same cluster that included isolates from broiler chicken, broiler meat and human clinical cases with unknown epidemiological relationship, all belonging to the ESBL-pESI-like-positive S. *infantis* clone previously described in Italy. Overall, these findings are of great concern, since this clone, has genetic traits mediated by the pESI-like plasmid of enhanced virulence, MDR and fitness in the intensive farming system, and it is often ESBL-producing. Additionally, we have demonstrated that this clone has the attitude to acquire additional transferable resistance to last-resort drugs like colistin. These characteristics inevitably lead to a further reduction of therapeutic options for *Salmonella* invasive infections in humans, transmitted through the food-chain. Our results highlight the need of implementation of risk-management strategies and actions to be taken in order to drastically reduce the amount of colistin used in broilers in Italy and reduce the overall prevalence of S. Infantis, within the Italian broiler chicken industry.

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Phylogeography of rabies virus isolated from cattle and horses transmitted by haematophagous bat Desmodus Rotundus in Brazil.

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Abstract

The correct identification of pathogens is essential as well as its transmission form. Epidemiological or statistical studies can identify the geographical dispersion of different types of pathogens. The phylogeography expresses the contemporary pattern of geographic distribution of an organism according to gene genealogies. The phylogeography is studied by methods that use the Bayesian Markov Chain Monte Carlo Method (MCMC), a statistical method that tests hypotheses and is available in BEAST package (Bayesian Evolutionary Analysis Sampling Trees), that allows various evolutionary models to be tested using a set of genetic sequences obtained over time in a geographic area. BEAST returns rooted trees and the dates of the trees of time can be converted into a keyhole markup language (KML) file suitable for viewing with Google Earth to provide a spatial diffusion of the genetic lineages. This summary shows results of a phylogeographic study using samples of the Rabies virus (RABV) circulates in an important transmitter of the disease, the vampire bat Desmodus rotundus, in an endemic area of the state of São Paulo, southeastern, Brazil. RABV display evolutionary and ecological dynamics on the same time scale reliable phylogeographic inferences can be obtained from molecular data. This study determines the dispersion over time and space of the RABV transmitted by D. rotundus. The obtained results demonstrate that since 50's two lineages of RABV transmitted by D. rotundus circulate in the area. One lineage was subdivided into two other lineages of virus resulting in a total of tree lineages. Each of one identified lineage had different direction in the country: south, north and west. In this way, the final results can aid epidemiological surveillance and also strategic planning for the control of rabies or other pathogens.
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Effect of pH on the in vitro activity of fluoroquinolones in combination with cephalosporins against multidrug-resistant *Escherichia coli.*

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Abstract

The present study was performed to investigate the effect of pH on the *in vitro* synergistic activity of floroquinolone (FQ) in combination with cephalosporin (CEPH) against selected strains of multidrug-resistant (MDR) *Escherichia coli* (*E.coli*). Broth microdilution testing was performed to determine minimum inhibitory concentrations (MICs) of the antimicrobials according to the guidelines of the Clinical Laboratory Standards Institute (CLSI). The Mueller-Hinton Broth medium used for fractional inhibitory concentration (FIC) tests was adjusted to pH values of 5.0, 6.0, 7.3, 8.0 with pH meter by dropping 1N HCl and 1N NaOH solutions separately after autoclaving. Fractional inhibitory concentration index/indices (FICi) of antimicrobials were determined using checkerboard method. Six of *E. coli* isolates were chosen for combination tests based on their resistance profile. The FICs of the FQ/CEPH combination were calculated at different pH values. FICs ranged from 0.38 to 8 for pH 5.0; from 0.16 to 8 for pH 6.0; from 0.03 to 1.03 for pH 7.3; from 0.03 to 0.3 for pH 8.0. The combination of FQ/CEPH was found to be synergistically effective at pH values of 8.0, 7.3, 6.0 and synergy rates were 5.0%, 83%, 50% and 16%, respectively. Antagonism was detected only for 3/6 (50%) of E. coli isolates at pH 5.0 and for 1/6 (16%) for pH 6.0. The results of this study showed that FQ/CEPH combination was more effective in alkaline medium than in acidic medium against resistant *E. coli*. Acknowledgements**:** TUBITAK (TOVAG-214O316)

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Vespa

MIC distribution, MIC50 and MIC90 in motile *Aeromonas Hydrophila* isolated from diseased fish.

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Abstract

Motile Aeromonads cause atypical furunculosis in freshwater fish. The infection is characterized by skin ulceration, hemorrhages, fin rot, and septicemia. *Aeromonas (A.) hydrophila* has gained much attention as the most common fish pathogenic motile *Aeromonas* species. To study the antimicrobial susceptibility, interpretative criteria (epidemiological cut off values) are available only for *A. salmonicida*, regarding florfenicol, ormethoprim-sulfadimethoxine, oxytetracycline and oxolinic acid. Moreover, diverse susceptibility tests are performed for *A. hydrophila*. The minimum inhibitory concentration (MIC) test is conducted using broth dilution or agar dilution technique. Aim of this study was to investigate the MIC distribution, calculating MIC50 and MIC90 in *A. hydrophila* strains isolated from fishes affected by furunculosis. 53 strains of *A. hydrophila* were identified in diseased fish, sampled from farms located in the northern and central regions of Italy. A commercial broth microdilution plate (Micronaut S; Merlin Diagnostics; customized plate) was used, according to CLSI guidelines. *A. salmonicida subsp. salmonicida* ATCC 33568 was used as control. The molecules, belonging to seven antibiotic classes, were as follows (the test range, µg/ml, is reported in brackets): ampicillin (0.0625-32), apramicin (0.25-64), colistin (0.015625-8), trimethoprim-sulfamethoxazole (0.0625/1.1875-16/304), aminosidine (0.25-32) gentamicin (0.0625-32), flumequine (0.25-32), enrofloxacin (0.015625-32), florfenicol (1-64), tetracycline (0.03125-16). From the obtained distribution of MICs values, MIC50 and MIC90 were calculated. MIC values below the tested range were recovered for enrofloxacin, florfenicol, and flumequine. On the opposite, MIC values above the tested range were found for colistin. MIC's distribution showed two distinct peaks for colistin and aminosidine. In this study, the MIC50 for trimethoprim-sulfamethoxazole and for tetracycline of pathogenic *A. hydrophila* strains, is in agreement with that obtained by Baron *et al.* (2017) in strains collected from the aquatic environment, whereas it is lower than the value obtained in other reports. Moreover, gentamicin MIC50 and MIC90 values (0.5 and 1 µg/ml, respectively) were equivalent to those recorded in the literature for Aeromonas spp. and, as previously reported, intrinsic resistance to ampicillin was observed (MIC>32 µg/ml).

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Minimal inhibitory and mutant prevention concentrations of enrofloxacin and marbofloxacin for *Escherichia coli* clinical isolates of rabbit.

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Abstract

There is an urgent need to develop medication regimens that prevents the selection of drug resistant bacteria. Several data supports the use of Mutant Prevention Concentration (MPC) instead of Minimum Inhibitory Concentration (MIC) to optimize dose and dosing intervals. The clinical applications of MPC were investigated quite extensively with different antimicrobial-pathogen-animal species combination. Nevertheless, investigations concerning pathogens for food producing animal species as rabbit are very low. Therefore, an in vitro study was performed to provide a preliminary representation of sensitivity to Enrofloxacin (ENRO) and Marbofloxacin (MARBO) of pathogenic Escherichia coli (E. coli) isolated from rabbits dead by colibacillosis. Fourteen pathogenic strains of E. coli were selected in two different industrial farms of South Italy. MICs and Minimum Bactericidal Concentrations (MBC) tests were performed as recommended by CLSI and NCCLS guidelines. MPC experiments were performed as previously described with minor modifications. Isolates were classified as susceptible, intermediate resistant or resistant according to the CLSI breakpoints for ENRO in chickens and turkeys. MARBO susceptibility breakpoints were those proposed for antimicrobial used in animals. The Mann-Whitney or Wilcoxon signed rank test were used for statistical comparisons. MICs of ENRO (0.015-64 mg/ml) and MARBO (0.015- 16 mg/ml) were widely distributed for both drugs and 36.7% of susceptible (MICENRO ≤ 0.25; MICMARBO ≤ 1), 28.6% of intermediate resistant and 36.7% resistant (MICENRO ≥ 2; MICMARBO ≥ 4) E. coli isolates were detected. No differences in bacteria sensitivity were found between the two selected farms. MBC values show very slight differences (equal or 2-fold dilution) compared to the corresponding MICs confirming the good bactericidal activity for both drugs. No significant differences in sensitivity to ENRO and MARBO were found in susceptible and intermediate resistant bacteria but MARBO demonstrated significant higher level of activity ($P<0.05$) in resistant E. coli. MPC values calculated for 4 susceptible and 3 intermediate resistant strains ranged from 0.5 to 1.0 mg/ml and from 0.25 to 0.5 mg/ml for ENRO and MARBO respectively. The calculated size of the Mutant Selection Index (MSI = MPC/MIC) was significantly lower ($P<0.05$) for MARBO compared to ENRO. Despite the limited number of tested isolates, quite a high presence of resistant strains were found and MARBO can be predicted to be less worrying against the selection of additional bacteria mutants. A therapeutic dosage revision is suggested to minimize the selection of resistant mutants and reduce the reservoir of resistant mutants.

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Antifungal susceptibility of *Malassezia pachydermatis* isolates from dogs to antimycotics and essential oils alone and in combinations.

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Abstract

Malassezia pachydermatis, a commensal yeast commonly found on the skin of healthy dogs is also often diagnosed in dogs with dermatitis. For the treatment of malasseziosis in dogs preparations containing mainly polyene and azole antimycotics are available. A combination of conventional antimycotics and essential oils (Eos) is presumed to improve the antifungal activities of the drugs to which the resistance has developed. Using the standard disc diffusion method M44-A2 (CLSI, 2009) with some modifications, the effectiveness of six antimycotics (fluconazole - FLU, itraconazole - ITR, miconazole - MCZ, clotrimazole - CLO, voriconazole - VOR and posaconazole - POS) and five essential oils (basil, oregano, mint, juniper and nutmeg) at concentrations of 20%, 10%, 5% and 0.5% against 18 clinical isolates of M. pachydermatis was tested. A 72-hour culture of M. pachydermatis isolates was used to prepare a suspension of inoculum containing 106 CFU/ml which was spread on the surface of the agar (Sabouraud dextrose agar supplemented with 0.1% Tween 80). Commercial antimycotic discs were placed on its surface afterwards. When testing the effectiveness of EOs, blank discs were impregnated with the individual concentrations of each EOs at amount of 15 µl. The antimycotic activity of the tested antimycotics was as follows: FLU - 83.3%, ITR - 50%, MCZ and CLO - 33.3%, VOR - 100% and POS 94.4%. Out of the tested EOs oregano EO was the most effective at concentrations of 20% and 10% showing 100% efficiency. The antifungal activity at 5% concentration was 55.6%. Basil EO was effective only on 3 isolates (16.7%) at a concentration of 20%. Mint EO inhibited the growth of 6 isolates (33.3%) at a 20% concentration. No antifungal activity was found with other concentrations of these oils. Juniper EO and nutmeg EO were not effective at either of the tested concentrations. In order to detect the synergistic effect of antimycotics and the essential oils, clotrimazole was chosen as the agent to which the isolates were the most resistant. From the EOs the synergistic effect of basil EO at 10% concentration and oregano EO at the 5% concentration was studied. While only 6 isolates (33.3%) were susceptible to clotrimazole alone and 12 strains (66.7%) showed resistance, 17 strains (94.4%) were susceptible and only 1 strain (5, 6%) was resistant to the combination of clotrimazole with basil EO. Similarly, the sensitivity of the isolates was increased in combination with oregano EO where 100% efficiency was found. This work was supported by the Slovak Research and Development Agency under the contract No. APVV-15-0377.

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Interspecies differences in antimicrobial drug pharmacokinetics in birds.

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Abstract

Enormous interspecies differences exist among bird species, due to their extremely diverse physiology. Huge interspecies dissimilarities occur in ADME processes and consequently in therapeutic effect of the different drugs. For rational antibacterial medicine use it is advisable to examine each active substance in all target bird species. The goal of our study is to compare the pharmacokinetics of a model substance (sulphachlorpyridazine sodium) in two poultry species. Clinically healthy domesticated chickens (*Gallus gallus domesticus*) and Guinea fowls (*Numida meleagris*) (Herbro Kft., Hernád, Hungary) were used in this study, five males and five females of each species. After one week acclimatization period 10 young adult birds of both species were administered intramuscularly in the chest muscle with sulphachlorpyridazine sodium (100 mg/kg bw.) once. Blood samples were collected before the treatment (0) and 20, 40, 60, 90, 180 and 300 minutes after the injection. Plasma levels of sulphachlorpyridazine sodium were determined by validated high-performance liquid chromatography (HPLC). Pharmacokinetic parameters were calculated by WinNolin 5.2.1. Pharmacokinetic values of sulphachlorpyridazine sodium in chickens were; Cmax=195.25±41.46 mg/l, Tmax=86.20±26.98 min, AUC0-t=52234.83±11030.12 min×mg/l, AUC0-∞ =67512.34±21321.34 min×mg/l, λz =0.0041±0.0018 1/min, t1/2=193.97±55.93 min, MRT=297.87±89.63 min, Vss=456.31±126.10 l, Cl=1.63±0.54 ml/min. Pharmacokinetic values of sulphachlorpyridazine sodium in Guinea fowls were; Cmax=263.57±58.31 mg/l, Tmax=62.45±35.79 min, AUC0-t=78067.27±15828.40 min×mg/l, AUC0-∞ =94889.67±26652.68 min×mg/l, λz=0.0039±0.0013 1/min, t1/2=189.62±55.42 min, MRT=305.34±73.65 min, Vss=331.29±71.41 l, Cl=1.14±0.33 ml/min. Statistical differences occurred between domesticated chickens and Guinea fowls in the following pharmacokinetic parameters; Cmax, AUC0-t, AUC0-∞, AUMC0-∞, Vss and Cl. Significant differences are shown in the elimination processes between the two bird species, in spite of that the investigated species are closely related to each other, they belong to the same family. Our results drew attention to the interspecies differences in bird species, the pharmacokinetics of each drug should be investigated in the target species to determine the safest and most effective dose, to prevent the irrational use of antibacterials.

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Novel antimicrobial efficacy against campylobacter bio-films using the natural plant compounds, trans-cinnamaldehyde, eugenol or carvacrol on food processing surfaces.

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Abstract

Campylobacter jejuni is the leading cause of human foodborne illness globally, and is strongly linked with the consumption of contaminated poultry products. Often, *C. jejuni* survives in the processing environment by forming biofilms, complex bacterial communities with increased resistance to disinfectants and antibiotics. Research to develop effective control strategies against *C. jejuni* biofilms has been limited. This study investigated the efficacy of three phytochemicals, trans-cinnamaldehyde (TC), eugenol (EG) or carvacrol (CR) in inhibiting or inactivating *C. jejuni* biofilms on common food contact surfaces. For the inhibition study, *C. jejuni* NCTC 11168 was grown either in the presence or absence (control) of sub-inhibitory concentration of TC (0.01%), EG (0.01%) or CR (0.002%) for 48 h. For the inactivation study, *C. jejuni* biofilms were exposed to the phytochemicals (0, 0.25, 0.5, or 1%) for 1, 5, or 10 min, and surviving biofilm-associated *C. jejuni* were enumerated. All the studies were conducted three times with duplicate samples. All phytochemicals reduced *C. jejuni* biofilm formation as well as inactivated mature biofilm on polystyrene and steel surfaces at 20°C and 37°C ($P<0.05$). The highest dose (1%) of TC, EG and CR rapidly inactivated biofilm within 10 min to below detection limit (1 Log CFU/mL) at 20°C on steel surface. The lowest dose (0.25%) of all phytochemicals reduced counts significantly (> 3 Log CFU/mL) when treated for 1 min at 20°C on a steel surface. The genes encoding for motility systems (flaA, flaB, flgA) were downregulated by all phytochemicals ($P<0.05$). In addition, the expression of stress response (cosR, ahpC) and cell surface modifying (waaF) genes was reduced by 0.01% EG. LC-MS/MS based proteomic analysis revealed that TC (0.01%), EG (0.01%) and CR (0.002%) significantly downregulated the expression of NapA protein (required for signaling pathway during oxidative stress). The expression of DnaK (chaperone protein) and bacterioferritin required for biofilm formation was also reduced by TC and CR. Scanning electron microscopy revealed disruption of biofilm architecture and loss of extracellular polymeric substances after phytochemical treatment. Results suggest that TC, EG, and CR could be used as a natural disinfectant for controlling *C. jejuni* biofilms.

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UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI SCIENZE VETERINARIE PER LA SALUTE, LA PRODUZIONE ANIMALE E LA SICUREZZA ALIMENTARE

Correlation between oral fluid and plasma oxytetracycline concentrations after intramuscular administration in pigs.

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Abstract

The effective treatment of bacterial diseases depends on many factors, including concentration of antimicrobials at the site of infection. In the respiratory and gastrointestinal tracts, oral fluid is one of the medium and secretion, where many pathogens can be located. Thus, the knowledge about the penetration of the drug in oral fluid may be used to avoid development of resistance in bacteria strains, because in oral fluid the selection of resistant strains of commensals can occur. Additionally, the antibiotics in food producing animals are analyzed and controlled in tissues. However, regarding the overuse of medicine in veterinary medicine, there is a strong need to find and implement an alternative to post mortem analysis of antibacterials. The ante-mortem, non-invasive methods for drug testing is oral fluid analysis. The objective of this study was to evaluate the penetration of oxytetracycline, as widely used tetracycline in swine treatment, into the oral fluid and to compare the oxytetracycline concentrations in plasma and oral fluid of pigs after a single, intramuscular (i.m.) dose of 20 mg/kg body weight of a long-acting formulation. The oxytetracycline was detectable both in oral fluid and plasma from 1 h up to 21 day after drug administration. The maximum concentration (Cmax) of drug with values of 4021 ± 836 ng/ml in oral fluid and 4447 ± 735 ng/ml in plasma was reached (Tmax) at 2 h and 4 h after drug administration, respectively. The area under concentration – time curve (AUC), mean residence time (MRT) and the elimination half-life (t1/2β) were respectively, 75613 ngxh/ml, 62.8 h, 117 h in oral fluid and 115314 ngxh/ml, 31.4 h, 59.2 h in plasma. The oxytetracycline concentrations remained higher in plasma for 48 h. After this time, oxytetracycline reached greater levels in oral fluid. The strongest correlations at 2 h and 504 h were observed. Concentrations of oxytetracycline were within the therapeutic levels for the most sensitive microorganisms in pigs (MIC around 0.5 µg/ml) for 48 h after drug administration, both in the plasma and in oral fluid.

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Vespa

Simultaneous determination of 45 antibacterial drugs in mushrooms - Agaricus bisporus by ultra-high-performance liquid chromatography tandem mass spectrometry.

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Abstract

The *Agaricus bisporus* is well known as the white button and common mushroom, and is one of the common commercial mushrooms. A. bisporus is also one of the most economically important mushroom in the world with a global crop exceeding 2 million tonnes. Possibility of using poultry manure and litter in the production of ground for cultivation of mushrooms is an important element of the eco-economy with environmental benefits. However, large-scale production of animals often involves the use of antibiotics to combat disease, unfortunately most of them are poorly absorbed and are excreted in unchanged form in manure. This fact causes antibiotic residues in high concentration to be present in manure and can be the cause of mushrooms ground antibiotic contamination and then the mushrooms as well. This resulted in the need to develop an analytical method that would enable monitoring of antibiotic residues in *A. bisporus*. A multi-residue and multi-class method was developed and optimized for the determination of 45 antibacterial compounds in mushrooms – *A. bisporus*. This method is based on liquid-liquid extraction with acetonitrile with addition of trichloroacetic acid and filtered through sodium sulphate, before the identification and quantification of the residues by ultra-high performance liquid chromatography triple quadrupole tandem mass spectrometry (UHLC–MS/MS). Satisfactory linearity was obtained for all compounds with regression coefficients greater than 0.99. The recoveries were in the range of 73 - 118%. Repeatability and intra-lab reproducibility were lower than 10 and 15%, respectively. The Limit of quantification (LOQs) was in the range of 1 - 10 µg.kg^{-1} and the Limit of detection (LODs) ranged from 0.5 – 2 µg/kg^{-1} for all analytes in the matrices. The proposed method can be successfully applyed to the determination of antibiotics in commercially cultivated common mushrooms.

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Vespa

Monitoring and control of AMR in products of animal origin in the Russian Federation.

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Abstract

Russian Federation develops measures to control AMR in line with internationally recognized approaches. "The National Strategy on Prevention of Antimicrobial Resistance Spread in the Russian Federation for 2017 - 2030" was approved by the Government in 2017. Among key points of this Program:

- data collection on volumes of antimicrobial circulation and application using special electronic information systems,

- harmonization of AST methods, development and implementation of the National Program of AMR Monitoring in the veterinary field,

- risk analysis of occurrence and circulation of resistance, including assessment of the main trends in the distribution of zoonotic antibiotic-resistant bacteria in livestock and veterinary medicine,

- definition of genetic determinants of AMR,

- development and scientific substantiation of recommendations for minimizing the spread of AMR and the safe use of antibiotics in veterinary medicine.

AST is performed using the internationally harmonized broth microdilution test. Bacteria and the number of isolates have been chosen in accordance with OIE recommendations. Isolates are taken from biomaterial of poultry, cattle, swine, reindeer and various kinds of food and feed. More than 30 antimicrobials from 12 classes were included in the program based on data on medical and veterinary importance as well as sales volumes in Russia. MIC are interpreted using EUCAST, CLSI and Russian epidemiological and clinical breakpoints. Data will be provided to internationals organizations, including OIE.

In 2017 year 366 isolates were identified: Campylobacter spp - 8, Salmonella spp - 102, E.coli - 127, Enterococcus spp - 129. Preliminary results showed multiresistant properties for Salmonella, Enterococcus and E.coli isolates. Epidemiological resistance for E.coli was observed to penicillins, cephalosporins, aminoglycosides, sulfonamides, tetracyclines and quinolones, for Enterococcus to tetracyclines, macrolides, glycopeptides, oxazolidinones, anzamycins, diaminopyrimidines. Genes of resistance for some isolates are investigated by whole-genome sequencing. Several large plasmids were discovered conferring simultaneous Salmonella resistance to penicillins, cephalosporins, aminoglycosides, sulfonamides and tetracyclines, similar to plasmids isolated in the USA and Italy. Salmonella resistance to fluoroquinolones was determined by chromosomal mutation.

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Assessment of marbofloxacin levels in intestinal contents of pigs to establish a PBPK model.

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Abstract

Fluoroquinolones, especially Marbofloxacin, are used in pigs for the treatment of several diseases. In this species, the administration is generally via parenteral route. It was clearly shown that fluoroquinolones are extensively excreted in the gastrointestinal tract, with concentrations largely higher than plasma concentrations for some compounds. We previously developed a PBPK model for colistin in pig and we added some compartments to describe the intestinal excretion of marbofloxacin. The goal of this study was (i) to develop and validate simple and reliable HPLC methods with spectrofluorimetric detection (FL) to quantify marbofloxacin and get robust data to (ii) built the intestinal tract part of the PBPK model. Validation of analytical methods (for small and large intestines' content) is based on the accuracy profile. Intestinal contents (duodenum, proximal and distal jejunum, ileum, proximal colon and distal colon) were obtained from 18 pigs that received marbofloxacin at a dose rate of 2 mg/kg/day during 3 days by IM route. After a liquid-liquid extraction 100 µl was injected in HPLC systems. Excitation and emission wavelengths for detection by FL were 299 nm and 507 nm respectively. Three series of 4 concentration levels within 500-20000 ng/g with 3 replicates were carried out on spiked intestinal contents. The statistical analysis was performed with the software e.noval® 3.0 with acceptability criteria of 20 %. The accuracy profile obtained allowed to determine a lower limit of quantitation of 530 ng/g for all compounds and all methods were validated. Compartments representing different segments of intestinal tract were added to our initial PBPK pig model. The PBPK analysis is performed with Monolix. The capacity of the model to predict intestinal level was assessed by using a Bland-Altman plot. Predictions are close to the observations data with 94.1 % of data within the 95 % confidence interval. Visual Predictive Check (VPC) are satisfying except for jejunum. The central trends revealed that only 3.09 % of observation are outliers i.e. a low number of observations is outside of 98 % of tolerance interval. In conclusion, analytical methods and the developed PBPK model are suitable. PD model can now be linked to intestinal compartments to study the antibiotic resistance with each segment bacterial populations.

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UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI SCIENZE VETERINARIE PER LA SALUTE, LA PRODUZIONE ANIMALE E LA SICUREZZA ALIMENTARE

Detection of ESBLs producing *Escherichia coli* isolated from dog faeces from La Plata city.

Lihuel Gortari[1], Andrea Buchamer[1], María Laura Marchetti[1], Daniel Buldain[1], Florencia Aliverti[1], Manuel Chirino-Trejo[2], Nora Mestorino[1,*]

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Abstract

The great increase in strains carrying extended spectrum beta-lactamases (ESBL) is a problem worldwide. Pets would have a possible role as reservoirs for ESBLs. Enterobacteriaceae are the mainly producers of ESBL, particularly Klebsiella pneumoniae and Escherichia coli. ESBLs are plasmid-mediated enzymes that hydrolyze penicillins, third generation cephalosporins and aztreonam, but are susceptible to beta-lactamase inhibitors (clavulanic acid). Escherichia coli among intestinal microbiome, has a role of resistance indicator bacteria. The surveillance of its resistance mechanisms is an important tool in the control of antimicrobials non-prudent use. Our objective was to detect ESBLs produced by E. coli strains isolated from pet and stray dogs of La Plata city, Buenos Aires, Argentina. Samples were collected using rectal swabs from 50 home dogs (HD) and 50 stray dogs (SD). Strains were stained by Gram and typified by biochemical tests. Susceptibility profiles for 20 antimicrobials was performed by standard Kirby-Bauer disk diffusion method, and the first ESBL disc screening was evaluated using cefpodoxime, ceftazidime, aztreonam, cefotaxime and ceftriaxone. For the ESLBs's confirmation, the Double-Disc Synergy Test was done: discs containing cetazidime, cefpodoxime were applied next to a disc with amoxicillin plus clavulanic acid (20 mm center to center). Positive result is indicated when the inhibition zones around any of the cephalosporin discs were augmented in the direction of the disc containing clavulanic acid. E. coli ATCC 25922 was used for quality control. There were multiresistant strains in both groups and their resistance profile includes one, two, three and more than four antimicrobials. Multiresistance occurrence was considerably higher in SD strains (42% of multiresistance to more than 4 antimicrobials). There were 8 % of strains suspected of being ESLBs producing among samples of HD and 36 % of SD. One of the strains ESBL suspected of HD (2%) and 11 (22%) of SD, were confirmed. Conclusion: ESBLs producing strains identification, is an essential tool to achieve efficacy in the antimicrobial therapy. It is important to know the development of antimicrobial resistance by monitoring not only animals at home, but also those homeless ones.

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Bromhexine effects on enrofloxacin penetration in bronchial chicken secretion.

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Abstract

Bromhexine (BROM) is a derivate of the Adhatoda vasica, plant used in some countries for the treatment of various respiratory diseases. It enhances the secretion of various mucus components by modifying its physicochemical characteristics, increasing mucociliary clearance. Furthermore, the co-administration of antibiotics with bromhexine amplified the actions of the antibiotic. Its use is approved intramuscularly or orally in calves, pigs and chickens at a rate of 0.5 mg/kg/day for 5 consecutive days. Our objective was to determine the possible increase of penetration of enrofloxacin (ENR) into bronchial secretions when combined with the fluidifying BROM. One hundred and twenty chickens were divided in two groups of sixty animals each: one group was treated with ENR alone and the other was treated with the ENR/BROM combination. Both formulations were administered at 10 mg/kg for five days. Plasma and bronchial secretions samples were obtained at different times during treatment and up to 24 h post-administration. The presence of ENR and its metabolite ciprofloxacin in all samples were determined by HPLC with fluorescence detection. Pharmacokinetic analysis was performed by non-compartmental methods with Phoenix® WinNonlin® 8.0, copyright ©2005-2017, Certara, L.P. ENR maximum plasma concentration (Cmax) obtained after ENR/BROM combination administration, was higher than the achieved after the administration of ENR only (0.77 µg/mL vs. 0.63 µg/mL, P=0.0332), and was achieved later (Tmax 105.75 h vs 90 h). Although no statistically significant difference was found when comparing the areas under the curve plasma concentration as a function of time (AUC) between both groups, it did obtain significant difference in the medium residence time (MRT 88.14 vs 80.66 h, P=0.0214). In bronchial lavage, greater differences were found after combination administration, a higher Cmax (0.90 vs 0.49 µg/mL, P=0.0344) was obtained more quickly (Tmax of 24 h vs 58.29 h, P=0.0082), with a higher bioavailability (AUC 59.50 vs 36.03 µg.h/mL, P=0.0457), but the MRT was shorter (55.10 vs 81.66 h, P=0.0139). The results showed that BROM facilitates the ENR penetration into the airways, achieving higher concentrations and faster than when given ENR alone, which would allow to quickly reach the predictors of antimicrobial efficacy (AUC/MIC) to attack the microorganisms responsible of respiratory infections in broilers.

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Rifaximin and melaleuca armillaris essential oil combinations against Staphylococcus aureus.

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[1] *Pharmacology and Toxicology, Veterinary Faculty. Universidad Nacional De La Plata, La Plata, Argentina*

Abstract

Rifaximin (RIF) is an antibiotic with high activity against Staphylococcus spp. However, it is necessary a prudent use to avoid resistance selection. Another antimicrobial resource choice is represented by the essential oils (EO). There is evidence that Melaleuca armillaris Sm. EO has antimicrobial activity. Our objective was to evaluate the existence of a possible synergistic effect between the EO and RIF at different pH conditions. EO was obtained by steam distillation of leaves and herbaceous branches. Susceptible S. aureus strains wild type (n=3) isolated from Holstein cows and S. aureus ATCC 29213 were used. The antimicrobial activity of RIF, EO and RIF/EO combination was evaluated by microdilution in broth using the checkerboard method. The concentration range was 256-0.007 µg/mL for RIF and 50-0.1 µL/mL for EO. The existence or not of synergism between both was determined by the FIC index (Fractional Inhibitory Concentration). Time kill assays were used to evaluate the antibacterial activity index E, defined as the difference between the Log10 values of the number of viable bacteria (CFU/mL) at initial time (nt-0) and at the end of the test (nt-24) according to: E= nt-24h - nt-0 (E= 0 bacteriostatic effect, E= -3 bactericidal effect, E= -4 virtual eradication effect). The concentrations evaluated corresponded to 1MIC (mixture with lower FIC), 0.5MIC, 2MIC, 4MIC and 8MIC maintaining the proportion of both compounds (dilution or concentration depending on the case). RIF had high potency (0.032 µg/mL) against S. aureus independently of the pH conditions. The MIC of EO was 25 µL/mL for ATCC 29213 and 12.5 mL/mL for wild types at pH 7.4. Both values decreased two-fold at pH 5.Combining RIF with EO, we found a synergic effect where the antibiotic was potentiated, particularly at pH 5 (MIC decreased 8-fold with 1.6 µL/mL of EO). A mix of 0.004 mg/mL of RIF and 12.5 mL/mL of EO allows reaching a virtual eradication effect (E= -4.0) against wild types strains at pH 7.4. Something similar occurs for reference strain. The acidification of media improves the EO/RIF activity. We can conclude that rifaximin is a potent antibiotic against S. aureus and does not require the use of adjuvants. However, these results are important findings for the treatment of staphylococcal infections of difficult resolution, because with the emergence of antimicrobial resistance of *S. aureus* to conventional antibiotics the treatment options for infections have become limited.

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Antimicrobial resistance of mastitis environmental pathogens.

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Abstract

Cow mastitis induces milk losses, lower milk quality, higher treatment costs and increased probability of premature culling and death. Mastitis pathogens can be classified as contagious pathogens (i.e. S. agalactiae, S. aureus and M. spp) and environmental pathogens (S. uberis, S. dysgalactiae, E. coli and Klebsiella spp), the latter being an indicator of poor management of the herd. The aim of this study was to evaluate the presence of environmental mastitis pathogens and their profile of sensitivity and resistance to antimicrobials in milk samples collected from 392 quarters of 98 lactating Holstein Fresian cows. Animals evaluation and milk sampling were done during the milking session. California Mastitis Test (CMT) score was performed for each quarter before the milking routine. Sterile milk samples were collected from each quarter for bacteriological examination from 176 (out of 392) CMT positive quarters. Fifty-three milk samples out of 176 CMT positive were positive at bacteriological examination. Environmental pathogens were found in 34 samples, 14 were positive to S. uberis and 20 to E. coli. Isolates were tested for antimicrobial sensitivity and classified as resistant, susceptible or intermediate according to CLSI standard. Isolates which showed intermediate susceptibility were classified as resistant. Four/20 (20%) E. coli isolates were susceptible to all antimicrobial tested, while resistance to 5 and 6 antimicrobials was observed in 20% (4/20) and 60% (12/20) of the isolates, respectively. None of S. uberis isolates was susceptible to all the tested antimicrobial while resistance to 6, 7, 8 and 12 antimicrobials was observed in 43.0% (6/14), 14.3% (2/14), 14.3% (2/14) and 28.6 % (4/14) of isolates, respectively. The presence of environmental pathogens and the patterns of resistance observed in this study suggest the importance of bacterial identification and sensitivity tests as criterion to choose the correct antimicrobial therapy.

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Live feed as source of antibacterial resistant bacteria in the culture of the red cusk eel Genypterus chilensis larvae.

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Abstract

Culture of red cusk eel *Genypterus chilensis* is currently considered a priority for Chilean aquaculture, but low larval survival rates mainly because of bacterial infections, prompted the need of a continuous use of antibacterial agents. It must be noted that use of antibiotics to treat live feed used in fish larval culture is frequent, so it is expected the occurrence of selection and spread of drug-resistant bacteria to reared fish larvae, precluding the success of a high-scale culture. The main aim of the study was to evaluate the role of live feed as source of antibacterial resistant bacteria in a commercial of the red cusk eel *Genypterus chilensis* larvae. Samples of rotifer and Artemia cultures, currently used as live feed in a commercial hatchery located in northern Chile were collected. Untreated and treated with florfenicol or oxytetracycline live feed cultures were sampled and total and resistant culturable counts were performed by a spread plate method. Rotifer and *Artemia* cultures exhibited high levels of culturable counts (7.1×10^7 CFU/g to 2.6×10^8 CFU/g, and 8.1×10^6 CFU/g to 6.1×10^7 CFU/g, respectively). Percentages of resistance to florfenicol and oxytetracycline ranged from 1.3% to 9.4% and 4.0 to 38.6%, respectively, in rotifer cultures, whereas in Artemia cultures varied from 5.1% to 19.1% and 22.1 to 43.0%, respectively. A number of 55 and 50 antibacterial-resistant strains was isolated from rotifer and *Artemia* cultures, respectively, and identified by 16S rRNA gene sequence analysis, observing a predominance of the *Vibrio* (83.6% and 76.0%) and *Pseudoalteromonas* (14.5% and 20.0%) genera. Isolates exhibited a high incidence of resistance to streptomycin (92%), oxytetracycline (85%), florfenicol (78%), co-trimoxazole (67%) and amoxicillin (52%), whereas resistance to flumequine (33%) and oxolinic acid (36%) were rather low. The high prevalence of multiresistant bacteria in live feed contributes to enrich the resistant microbiota of reared fish larvae, thus proper management strategies appear to be highly necessary to prevent future antibacterial therapy failures. Study supported by grant 1171772 of CONICYT, Chile.

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Multidrug resistance *Escherichia coli* harboring mcr-1 and blaCTX-M isolated from swine of Argentina.

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Abstract

The emergence and prevalence of colistin and extended-spectrum β-lactamases (ESBL)-*Escherichia coli* in food-producing animals are global public health concerns. In veterinary medicine, colistin and third-generation cephalosporins has been used through prophylactic or metaphylactic practices. In Argentina, colistin has been widely used in swine farms for many years to prevent enterobacterial infections and a third-generation cephalosporin are used to treat respiratory diseases, lameness and enteric diseases. The aim of this study was to investigate the presence of *mcr-1* and *bla*CTX-M genes in *E. coli* isolated from diarrheic and healthy pigs from 50 pig farms, located in the most swine-dense area of Argentina since 2004 to date. Antimicrobial susceptibility was evaluated by disk diffusion according to CLSI-M100S 27th ed. guidelines. Resistance and virulence genes were detected by PCR as previously described. For retrospective study, a total of 290 toxigenic and non-toxigenic *E. coli* isolated from diarrheic and clinically healthy pigs from 46 swine units were included. The isolates were originally collected and archived as part of other research and surveillance projects. Total *E. coli* were negative for the *mcr-1* gene and only 4 non-toxigenic *E. coli* (1.48%) carried the *bla*CTX-M gene and showed to be multiresistant. In 2017, 31 fecal samples from diarrheic piglets (2-3 days old) and healthy fattening pigs from 5 pig farms were incubated in buffered peptone water at 37°C overnight. Thirty µl of each enriched cultures were inoculated on either MacConkey agar plates containing 4 µg/ml cefotaxime or 2 µg/ml colistin. Thirty seven *E. coli* were obtained and 5 of them showed to be toxigenic (ETEC). Twenty *E. coli* (54.1%) harbored *bla*CTX-M, 9 (24.3%) *mcr-1*, 4 (10.8%) *mcr-1* plus *bla*CTX-M, 2 (5.4%) *bla*CIT, 1 (2.7%) *mcr-1* plus *bla*CIT and 1 (2.7%) *bla*PER. *E. coli* isolates showed non-susceptibility to: ampicillin (100%), tetracycline (81%), cefotaxime (76%), nalidix acid (76%), chloramphenicol (59%), minocycline (54%), ciprofloxacin (53%), TMS (38%), and gentamicin (19%); but were susceptible to: amikacin, and imipenem. Multiresistance (resistance to ≥ three classes of antimicrobial agents), was present in 27 (73%) isolates. In Argentina, *mcr-1* were previously reported in *E. coli* clinical strains isolated from inpatients and from healthy poultry. More prudent uses of antimicrobials in general are necessary, as well as the implementation of international measures to control zoonotic pathogens and limit the global emergence of these resistance traits.

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DIPARTIMENTO DI SCIENZE VETERINARIE PER LA SALUTE, LA PRODUZIONE ANIMALE E LA SICUREZZA ALIMENTARE

Beta-lactam antibiotics do not select for resistance equally: the rational behind their ranking.

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Abstract

The impact of beta-lactam antibiotics on the development of beta-lactam resistance was assessed. The resistance level of mutants recovered after 30 passages on sub-inhibitory concentrations of amoxicillin (AX), cefalexin (CX), amoxicillin+clavulanic-acid (AMC) and ceftiofur (CU) was compared in order to define which antibiotic selects for resistance more readily and the potential clinical impact of the acquired resistance. Five isolates, including 3 wild type strains, of *Salmonella typhimurium*, *Escherichia coli*, and *Klebsiella pneumoniae*, 1 TEM-1 producing *E. coli* and 1 CTXM-15 ESBL-producing clinical isolates were subjected to 30 serial passages on sub-inhibitory antibiotic concentrations of each drug using the Szybalski gradient method. Antibiotic MICs were determined by E-test and agar dilution. AmpC overproduction in *E. coli* mutants was evaluated by the disk diffusion method performed on Muller Hinton Agar alone and supplemented with 250 mg/L of cloxacillin. Efflux hyperexpression was sought by determining the MICs of the drugs in the absence and presence of 32 mg/L of PAβN. *Amp*C, *bla*TEM, *bla*SHV, *bla*CTX-M and their promoter regions were sequenced. OmpC, OmpF and OmpK36 porins genes were screened by PCR. AX selected for mutants that remained low-level resistant to AX and CX and susceptible to CU and AMC. After selection on AMC plates, the resistant mutants were resistant to AX and CX and susceptible to CU whilst the CX mutants were low-level resistant to AX, high-level resistant to CX and low-level susceptible or resistant to CU. Under CU, mutants exhibited a decreased susceptibility to AMX, a marked resistance to CX and a varying susceptibility or resistance to AMC and CU. Increased MICs involved cephalosporinase overproduction (*E. coli*) and/or decreased accumulation. No ESBL was generated. In contrast, under AMC exposure a mutation in the TEM gene of the *E. coli* TEM-1 producing organism was observed leading to the emergence of an Inhibitor Resistant TEM genotype. AX essentially selected for its own resistance. In contrast, cephalosporins selected for mutants with significant β-lactam resistance, including broad-spectrum cephalosporins. Our data argue for maintaining aminopenicillins as an empirical therapy for common infections, narrow-spectrum cephalosporins and AMC as alternatives and broad-spectrum cephalosporins as last resort antimicrobials.

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UNIVERSITÀ DEGLI STUDI DI MILANO
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Monitoring the evolution of E. coli CTX-M-1 ESBL under the selection pressure of various beta-lactams antibiotics: an in vitro study.

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Abstract

Whilst it is well known that antimicrobial agents have an impact on human intestinal flora together with selection and dissemination of multidrug resistant organisms, their long term role when at sub-inhibitory levels in the gut or in the environment, in the maintenance/loss of bacterial resistant determinants is however less clear. In this study, we evaluated 3 antimicrobial agents used in both human and/or veterinary settings (amoxicillin, co-amoxiclav and ceftiofur) on CTX-M-1 ESBL producing clinical strains of *E. coli* from human and animal origins. The effect of each drug on the maintenance or frequency of loss of bacterial plasmid harboring the resistant genes was evaluated by real-time quantitative PCR (qPCR). Two clinical strains of CTX-M1 ESBL producing *E. coli* (one from swine origin & 1 from hospital origin) were used in this study. The strains have been fully characterized previously at the molecular level. They were exposed to serial passages in liquid broth of sub-inhibitory levels (0.05 mg/l and 0.5 mg/l) of each of the 3 antibiotics. Two control tests representing each of the 2 clinical strains exposed in parallel to serial passages in an antibiotic free media were also used. All strains were grown overnight in a 10 ml broth culture. All cultures were standardized to inoculums of 106CFU/ml, then, 1ml of each culture was used to subculture three 9 ml broth tubes containing one antibiotic each. The broth culture were then incubated with shaking at 37°C for 8 hours and then subjected to 2 passages daily until 60 passages. A quantitative real time PCR (qPCR) was applied to monitor over time the loss of the plasmid harboring the ESBL in the host cell backgrounds. The quantitative corresponding real time curves were compared between each antibiotic together with the control test (strain under no antibiotic selection pressure). The results demonstrated that after 60 passages, amoxicillin had the closest effect to the antibiotic free media resulting in the loss of nearly half of the CTX-M genes initially present in the bacterial population followed by co-amoxiclav. Interestingly, ceftiofur exposure not only maintained the resistant determinants in the bacterial population, but resulted in an increase in copy number of the CTX-M genes in the bacterial culture. Our results demonstrated differential selection pressures exerted by sub-inhibitory levels of amoxicillin, co-amoxiclav and ceftiofur on the maintenance by *E. coli* isolates of the CTX-M-1 plasmid mediated ESBL.

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Epidemiology of gram-negative bacteria isolated from marine wildlife of the Aquitaine coast in south west of France.

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Abstract

Infections caused by antibiotic resistant gram-negative bacteria are becoming increasingly prevalent and represent nowadays a serious threat to public health worldwide. Multi-drug resistant (mdr) gram-negative bacteria have been confined to hospitals but from 2000s they have been reported in community patients and more recently from animals and the environment. In addition, there is a lack of new active antimicrobial agents against mdr gram-negative bacteria on the market. The aims of this study were to determine the prevalence of mdr bacteria in the marine environment together with searching for their potential reservoirs. This prospective study was carried out between April 2017 and June 2018. 300 samples were recovered (numerous fish species deep sea water, algae, sand…) from the Aquitaine littoral (south west of France). By mean of a combination of culturomics and metagenomics approaches, we have identified the microbiota of the specimen collected. The identification of the strains growing by culture was carried out by mass spectrometry (maldi-tof). Antibiograms according to EUCAST guidelines were carried out on all gram-negative bacteria in addition to multiplex pcr of the most clinically significant resistance determinants (blatem, blashv, blactx-m, blaimp, blavim, blandm, blaoxa, mcr…). The prevalence of clinically significant species of gram negative organisms was as follow: vibrio. spp 42%, E. Coli 5%, Enterobacter. spp 3%, K. pneumonia 2%, Serratia. spp 3%, pseudomonas. spp 9 %, proteus 1% and 35% of environmental bacteria amongst which 23% were unidentified by the maldi-tof. No mdr bacteria were detected. Indeed, the vast majority had a wild type profile. This study reports that mdr bacteria are not present in deep sea marine fauna/flora. This data is reassuring and highlights the need to maintain efforts in order to preserve this environment from contamination by mdr organisms. More studies are needed to confirm our findings.

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Antimicrobial resistance changes in *E. Coli* isolated from raptors in a center for injured wild animals during recovery.

Patrizia Nebbia[1,*], Mitzi Mauthe Degerfeld[1], Giuseppe Quaranta[1], Andrea Dogliero[2], Renato Lofiego[1], Patrizia Robino[1]

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Abstract

E. coli is an usual inhabitant of the avian gut and can easily acquire and transfer resistance genes. Commensal strains isolates from wild birds can be used as indicator of changes in antimicrobial resistance in animals that usually are not treated with antibiotics, but nevertheless they suffer the presence of anthropogenic activities. The aim of this work is to evaluate the effect of recovery/hospitalization on the antimicrobial resistance in fecal *E. coli* isolated from raptors submitted to the 'Centro Animali Non Convenzionali (C.A.N.C.)' of the Department of Veterinary Sciences (Turin University, Italy). This center takes care of injured wild animals, with the goal to return them into the wild, and is also involved in projects concerning the protection and conservation of some endangered species. Main anamnestic data (e.g. sex, age, reason for admission, antimicrobial use during hospitalization) were registered at the entrance of animals. Stool samples from 28 birds were collected at admission time (on day 0: T0) and during recovery (on day 7; T1) for each animal, with a total of 56 isolates of enteric *E. coli.* Identification of *E. coli*, ESBL production and antibiotic sensitivity tests were carried out following standard methods. The antibiotics panel included antimicrobial used in both veterinary and human medicine. Data were analyzed by descriptive statistics. Antimicrobial sensitivity tests carried out at the admission (T0) showed that 13 (46%) animals were infected with multidrug resistant strains and 2 birds were colonized by ESBL isolates. During the recovery (T1), ESBL *E. coli* were acquired in 2 animals (7.1%) and appearance of multidrug-resistance was observed in 8 birds (28.6%). To the best of our knowledge, this is the first report about changes associated to recovery in avian fecal *E. coli* comparing antibiotic resistance profile on the day of admission and after one week of permanence. Our work confirms data obtained on hospitalized domestic animals (e.g. dogs and horses). This finding is relevant because, in the routine of veterinary recovery of wild birds, most of the animals are committed for not less than two weeks.

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Fast and simple LC- MS/MS method for the analysis of tetracycline antibiotics in poultry feathers.

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Abstract

Tetracyclines are widely used in poultry production for the treatment of many bacterial diseases. However, overuse of medicines in chickens may contribute to a risk of their residues in food, as well as to bacteria resistance development. Under the EU official monitoring programs, tetracyclines are controlled in tissues of food producing animals. However, regarding the excessive use of drugs in animals, there is a strong need to find an alternative to post-mortem analysis of antibiotics. The use of feathers, as an unconventional matrix, enables control of birds' treatment during breeding. Thus, a fast and simple analytical method for the determination of seven tetracyclines (oxytetracycline, 4-epioxytetracycline, tetracycline, 4-epitetracycline, chlorotetracycline, 4-epichlorotetracycline, doxycycline) in poultry feathers has been developed. An extraction was performed using 5% trichloracetic acid. Samples were cleaned up by filtration using PVDF filters. For the determination of tetracyclines a liquid chromatography – tandem mass spectrometry (LC-MS/MS) method was used. Chromatographic separation was achieved on Luna C18 analytical column using mobile phase consisting of 0.1 % formic acid and 0.1 % formic acid in acetonitrile, within a total run time of 10 min. The method was validated according to the requirements of European Commission Decision 2002/657/EC. Good performance characteristics were obtained for recovery, linearity, precision, specificity, decision limits (CCα) and detection capabilities (CCβ). The procedure was satisfactorily sensitive with detection limit LOD = 2 µg/kg and the limit of quantification LOQ = 5 µg/kg. Poultry feathers analysis allows to distinguish registered and off-label use of antibiotic treatments. The developed method can be successfully applied in the control of tetracyclines administered in chickens. The application of presented procedure, should persuade more prudent and reasonable use of medicines in poultry farms.

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Injection site dependence of pharmacokinetics of antimicrobials in goose

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Abstract

In vertebrates, except for mammalian species, kidneys have a portal circulation system. Thereby, elimination of drugs injected to the lower body parts (leg or caudal musculature) could be considerably different from that injected to the chest. The goal of our study is to determine the bioequivalence of the model substance; sulphachlorpyridazine sodium, using two different administration sites in geese. Clinically healthy geese (*Anser anser domesticus*) were used in this study. After one week acclimatization period 10 adult mixed gender geese were divided into two groups, and a single dose of 100 mg/kg body weight sulphachlorpyridazine sodium was administered either into the breast muscle of five geese or into the hindlimb of other five. Two weeks later, according to the crossover study design, the injection sites were swapped. Blood samples were collected before the treatment (0) and 20, 40, 60, 90, 180 and 300 minutes after the injection. Plasma levels of sulphachlorpyridazine sodium were determined by validated high-performance liquid chromatography (HPLC). Pharmacokinetic and bioequivalence analysis were performed by WinNolin 5.2.1. Bioequivalence was pronounced when the 90% confidence interval of AUC0-t and Cmax parameters were within 0.8 and 1.25. Following the chest injection Cmax and AUC0-t values were 206.00±28.84 mg/l and 59218.87±9845.65 min×mg/l, following the leg injection Cmax and AUC0-t values were 175.70±37.06 mg/l and 52616.67±12610.36 min×mg/l, respectively. The Frel value was 0.89. Analysis of bioequivalence between the two injection sites showed that 90% confidence interval for AUC0-t were outside of conventional bioequivalence range of 80 % and 125 %. Our results drew attention to the potential differences in the bioequivalence of the active substances between intramuscular injection sites. It is especially true in bird species, because of their anatomy; the renal portal circulation accelerates the elimination of the drug injected to the leg muscle. In geese the breast muscle is better choice for intramuscular administration to achieve therapeutic concentration. This aspect should also be considered in other bird species and also in reptile species because of their similarity in kidney anatomy.

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Pharmacokinetics of tylosin tartrate in rapidly growing male turkeys.

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Abstract

Tylosin tartrate is a time-dependent macrolide antimicrobial commonly used in poultry. There is evidence that intensive growth is associated with decrease in drug clearance, which may differentiate drug efficacy in birds of different ages. The aim of the study was to investigate the effects of growth on the pharmacokinetics (PK) of tylosin tartrate in turkeys. Tylosin tartrate was administered intravenously (*iv*) at a single dose of 10 mg/kg or orally (*po*) at a single dose of 50 mg/kg to growing male BUT9 turkeys (n=10 for each route) four times: at the age of 5, 9, 12 and 15 weeks. During this time, the turkeys have grown from 1.5 kg to 15 kg of body weight. Blood samples were collected and plasma tylosin concentrations were assessed by HPLC-UV. Pharmacokinetics were calculated based on non-compartmental approach and differences among age groups assessed using Kruskal-Wallis test ($p<0.05$ considered significant). The area under the curve (AUC) after *iv* drug administration has significantly increased from 2.73±0.63 to 5.23±1.20; 6.69±1.49 and 7.12±2.32 mg×h/l, and the total body clearance decreased from 3.86±0.83 to 2.05±0.61; 1.58±0.39 and 1.56±0.50 l/h/kg, in 5-; 9-; 12- and 15-week-old turkeys, respectively. Mean residence time (MRT) increased from 0.21±0.05 to 0.22±0.07; 0.26±0.07 and 0.35±0.10 h. Similar increase was seen for the elimination half-life but volume of distribution did not show significant age-dependent correlation. AUC after *po* administration was 2.42±1.09; 2.10±0.97; 2.86±1.64 and 5.30±3.14 mg×h/l in 5-; 9-; 12- and 15-week-old turkeys, respectively. MRT increased from 1.81±0.36 to 2.70±0.59; 2.56±0.44 and 3.12±2.27 h. These parameters, as well as the elimination half-life did not show clear age-dependent differences. Bioavailability ranged from 8.0 to 17.7% without any obvious relation to age. After oral administration, the concentration of 0.05 µg/ml (MIC for *Mycoplasma gallisepticum*) was maintained for 4.75; 5.7; 5.8 and 8 h in turkeys of respective age groups. It is concluded that due to significant age-dependent changes in tylosin PK, young turkeys may require more frequent dosing in order to obtain similar clinical efficacy as in older birds.

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Context sensitive pharmacokinetics of cephalothin after intravenous administration in anesthetized dogs undergoing ovariohysterectomy by nonlinear mixed-effect modelling analysis.

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Abstract

Cephalothin (CPH) is a first generation cephalosporin indicated for antimicrobial prophylaxis of non-contaminated surgery. The recommended dose regimens are based on pharmacokinetic data obtained from awake animals, in which anesthesia or surgery effects are not present. Since CPH is eliminated by renal excretion, and it is known that anesthesia and surgery could affect the cardiac output, renal blood flow and glomerular filtration rate, pharmacokinetics of CPH may be context-sensitive when these factors are present. Sixteen healthy female dogs were included in the study. Six dogs were randomly assigned to a pharmacokinetic study of CPH by IV route without anesthesia and surgery. Moreover, the other ten dogs undergoing ovariohysterectomy, with anesthetic risk ASA I, were included in the pharmacokinetic study of CPH after IV administration under anesthesia and surgery conditions. CPH was administered IV at a dose of 25 mg/kg in both pharmacokinetic studies. Blood samples were collected at different time points until 12h. All animals finished the study and surgery without complications. CPH quantification in serum samples was determined by high performance liquid chromatography with UV detector (HPLC/uv). Concentration vs. time data was analyzed with a nonlinear mixed-effect modelling using Monolix (Lixoft, Batiment D, Antony, France), using the Stochastic Approximation Expectation-Maximization (SAEM) algorithm. A bi-compartmental pharmacokinetic model with a combined error model was selected. The pharmacokinetic parameters estimated by the model were Cl (clearance of the central compartment), V1 (volume of distribution of central compartment), Q (inter-compartmental clearance) and V2 (volume of distribution of peripheral compartment). The following covariates were included in the model to evaluate its effect on pharmacokinetics of CPH: age, weight and anesthesia/surgery conditions. The covariates were included in the model if showed statistical significance ($p<0.05$), reduced the Inter Individual Variability (IIV) and reduced 2X log-likelihood, Akaike Information Criteria and Bayesian Information Criteria values. After analysis, Anesthesia/surgery significantly reduced Cl, weight reduced Cl and V1 and age reduced V2, and were retained in the final model. Scatter plots of population/individual predicted vs. observed concentrations, population/individual weighted residuals vs. predictions/time, showed residuals little biased and uniformly distributed around and predictive value.

The final covariate model is described in the general form: Parameter = $\theta pop * Cov\beta\theta * e\eta\theta$

Where θpop is the population parameter estimation, $\beta\theta$ is the covariate parameter and $\eta\theta$ is the IIV.

The main findings of this study were that CPH pharmacokinetics are context-sensitive, and anesthesia/surgery conditions reduce the elimination of the drug, determining a higher permanence in the body. This fact is of therapeutic importance, since CPH is a time-dependent bactericidal action, and T>MIC is the main PK/PD parameter to assess antimicrobial efficacy.

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Pharmacokinetic analysis of cefquinome after intravenous and intramuscular administration in goats with different physiologic states by Nonlinear Mixed-Effect Modelling.

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Abstract

The objective of this study was to evaluate the effect of different physiologic states on the pharmacokinetics of cefquinome in adult goats (2 mg/kg), administered by intravenous and intramuscular administration, by nonlinear mixed-effects model. Eighteen healthy adult goats: 6 non-pregnant (NP), 6 pregnant (P) and 6 in the middle of the lactation period (L), were included in the study. The time of gestation in group P was 12.83±1.17 weeks. Milk production of animals in group L was 1.48±0.48 L/day. Cefquinome sulphate (CFQ) was administered IV or IM at a dose of 2 mg/kg, following a cross over design, with a washout period of 10 days. Blood samples were collected at different time points until 48 h for both routes. CFQ quantification in serum samples was determined by high performance liquid chromatography with UV detector (HPLC/uv). Concentration vs time data was analyzed with a nonlinear mixed-effect modelling using Monolix (Lixoft, Batiment D, Antony, France), using the Stochastic Approximation Expectation-Maximization (SAEM) algorithm. A bi-compartmental pharmacokinetic model with a combined and proportional error models were selected for IV and IM routes, respectively. The physiological covariates age, weight, non-pregnant, pregnant and lactating states, were evaluated in order to determine its effect on the estimated pharmacokinetic parameters Ka (absorption constant; only for IM route), Cl (clearance of the central compartment), V1 (volume of distribution of central compartment), Q (inter-compartmental clearance) and V2 (volume of distribution of peripheral compartment). The covariates were included in the model if showed statistical significance ($p<0.05$), reduced the Inter Individual Variability (IIV) and reduced 2X log-likelihood, Akaike Information Criteria and Bayesian Information Criteria values. After analysis, gestation significantly reduced Cl and Q values, since non-pregnant state reduced V1 for IV administration. Moreover, for IM route, only gestation was retained in the final model, and significantly reduced Cl and Q. Scatter plots of population/individual predicted vs. observed concentrations, population/individual weighted residuals vs. predictions/time, showed residuals little biased and uniformly distributed around and predictive value.

The final covariate model is described in the general form: Parameter = $\theta pop * Cov\beta\theta * e\eta\theta$

Where θpop is the population parameter estimation, $\beta\theta$ is the covariate parameter and $\eta\theta$ is the IIV.

The results of this study may be explained in terms of higher volume of distribution due to a higher total body water content reported in advanced gestation in many species, which could determine a higher distribution of CFQ to the extracellular fluids, resulting in lower Cl and higher V1 values.

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Effect of medium culture on pharmacodynamic effect of marbofloxacin against coagulase negative staphylococci isolated from goat mastitis.

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Abstract

Typically, in vitro pharmacokinetic/pharmacodynamic (PK/PD) models, used to evaluate PK/PD endpoints, are carried out using broth as a culture medium, as well as for the determination of MIC. It is known that different culture mediums could affect the MIC values compared to broth. The objective of this study was to evaluate the effect of milk when it is used as medium culture on pharmacodynamic parameters of marbofloxacin (MFX) against coagulase negative staphylococci (CNS) isolated from goat mastitis, in an in vitro static-PK/PD model. Thirteen strains of CNS isolated from goat mastitis were included. Time-kill curves were conducted using goat milk or Mueller Hinton broth as culture medium in tubes supplemented with MFX in order to achieve different AUC24/MIC values, based on previous MIC values determined by microdilution method on Mueller Hinton broth: 0h (control), 3h, 12h, 24h, 48h, 96h and 192h. The area between the control growth and time-kill curves (IE) was used to quantify the antimicrobial effect of each AUC24/MIC. Then, AUC24/MIC vs. IE data was analysed by a nonlinear mixed-effect modelling with Monolix program (Lixoft, Batiment D, Antony, France). A Emax model with constant baseline and constant error model was selected. The Emax model is described below:

$E = S_0 + ((E_{max} C^{\gamma})/(C^{\gamma} C_{50}^{\gamma}))$

Where S_0 is the baseline effect; E_{max} is the maximal effect; C is AUC24/MIC value; C_{50} is the AUC24/MIC value that produces 50% of E_{max} and γ is a sigmoidicity factor. Culture medium was included in the analysis as a covariate, in order to study its effect on the model parameters. Covariate were retained in the final model if showed statistical significance ($p<0.05$), reduced the Inter Individual Variability (IIV) and reduced 2X log-likelihood, Akaike Information Criteria and Bayesian Information Criteria values. The results of the analysis showed that using milk as culture medium reduced C_{50} (drug potency) and γ, without significant effect on the other parameters, that is in concordance with higher MIC values reported in milk. Scatter plots of population/individual predicted vs. observed concentrations, population/individual weighted residuals vs. predictions/time, showed residuals little biased and uniformly distributed around and predictive value. This findings suggest that PK/PD endpoints should be higher compared with those determined in broth for mastitis produced by CNS.

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UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI SCIENZE VETERINARIE PER LA SALUTE, LA PRODUZIONE ANIMALE E LA SICUREZZA ALIMENTARE

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Carriage of extended spectrum β-lactamase and AmpC-producing enterobacteriaceae (ESBL/AmpC-E) in petting zoos in Israel: a zoonotic hazard?

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Abstract

We aimed to investigate the prevalence, molecular epidemiology and risk factors for ESBL/AmpC-E carriage in petting zoos animals, since this population is in close contact with children. A prospective cross-sectional study (December 2016-May 2018) was performed in 8 petting zoos. ESBL/AmpC carriage was determined in two body sites: (i) gut (feces from 228 animals) and (ii) fur/skin/feathers (60% of animals). Samples after enrichment were plated onto CHROMagarESBL plates and sub-cultured to obtain pure cultures. ESBL and AmpC-production was determined according to EUCAST standards. Bacterial species identification and antibiotic susceptibility profiles were assessed using Vitek-2. Bacterial sequence types were determined using Multi-Locus Sequence Typing. ESBL and AmpC genes (CTX-M, SHV, TEM, CMY-2) were identified via PCR and sequencing. Owners' questioners were reviewed for risk factor analysis (SPSS). ESBL/AmpC-E carriage rate was 12% (n=28/228), with 35 recovered bacteria; 74% from fecal samples (n=26/35) and 23% from skin/fur/feathers (n=9/35). Thirteen isolates were both ESBL and AmpC-E. Overall, the main species was *Enterobacter cloacae* (52%), followed by *Escherichia coli* (31%), *Citrobacter freundii* (11%), *Citrobacter brakii*, and *Enterobacter amnigenus* (3% each). ESBL genes included CTX-M-1 group (17%), SHV-2 (9%), CTX-M-9 group, SHV-31 and SHV-12 (4% each), and 20% of the AmpC-E were CMY-2-positive. Eight *E. cloacae* sequence-types were identified: ST750 (2 animals from the same petting zoo) and seven single isolates sequence types- ST350, ST557, ST170, ST102, ST112, ST182 and ST511. Six *E. coli* sequence type: ST656 (4 animals from one petting zoo), ST648 (2 animals from one petting zoo), ST127 (2 animals, from one petting zoo) and 3 single isolates sequence-types ST4981, ST2521 and ST224. In a univariate analysis, ESBL/AmpC-E carriage was associated with antibiotic therapy (p=0.038 and p=0.011 for gut and skin/fur/feathers, respectively) and with petting permission policy (p=0.023). In a logistic regression model, antimicrobial therapy was identified as a risk factor (OR=7.34). Our findings demonstrates the diverse potential reservoir of ESBL and AmpC-E in petting zoos and possible intra-zoo transmission. The occurrence of resistant pathogens in petting zoos with high contact with children is alarming.

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UNIVERSITÀ DEGLI STUDI DI MILANO
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Single-shot perioperative antimicrobial prophylaxis in equine colic surgery – 5 cases.

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Abstract

Antimicrobial prophylaxis is an important measure for preventing surgical site infections (SSIs). At present, no reviewed guidance for perioperative antimicrobial prophylaxis in equine colic surgery is available. In Germany, a combination of penicillin and gentamicin (34.8 %) followed by penicillin (21.7 %) are among the most commonly used antibiotics to prevent postoperative infections in these horses. In human medicine, perioperative antimicrobial prophylaxis should not exceed 24 h and clean/contaminated surgeries in humans such as abdominal surgery are commonly carried out with a "single shot" perioperative antimicrobial prophylaxis including an intraoperative repetition dose after two half-lives of the antibiotic agent if necessary. However, a single-shot protocol in equine colic surgery has not been examined yet. Here we report on five cases of horses presented for colic surgery which had received a single-shot of penicillin (22000 IU/ kg BW, repetition dose after 80 min during surgery) and gentamicin (6.6 mg/kg BW, no repetition dose) applied 30 min prior to the initial incision. After surgery, each horse was dressed with a sterile bandage. At day three post operatively, the belly bandages were aseptically changed and all were removed at day five. Surgical sites were scored using a 0-9 point scoring format based on 3 assessments (exudation, swelling, suture dehiscence) each graded from 0 (best) to 3 (worst) at day 3, 5 and 10. At day three, one horse showed mild serosanguinous exudation (1 point), while the other horses (4/5) showed no signs of either exudation, dehiscence or swelling (0 points). However, one horse was euthanized due to postoperative ileus at day 3. At day 5, 3/4 horses showed serosanguinous exudation, one of them showed swelling (1 point) additionally. None of the horses showed dehiscence. One horse was discharged at day 9 (0 points). At day 10, one horse showed discharge, swelling and dehiscence (1/2/1; 4 points). The other two horses had final score of 0 points. As a preliminary result, single shot perioperative antimicrobial prophylaxis seems to be a possible choice for equine colic surgery, but further research is warranted.

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Antimicrobial resistance patterns of *Escherichia coli* isolated from canine urinary samples submitted to IZSLER diagnostic laboratories in 2016–2017.

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Abstract

Bacterial urinary tract infections (UTI) represent a common reason requiring veterinary care and *Escherichia coli* is the most frequently isolated pathogen. Early diagnosis and prompt antimicrobial therapy are recommended when clinical signs of a UTI are present. Submission of urine samples for culture and sensitivity testing is the best practice approach to diagnosis. Two hundred-thirty strains of *E. coli* isolated from January 2016 to December 2017 from urine samples belonging to as many dogs suffering of UTI were tested for the antimicrobial susceptibility using the Kirby-Bauer disc diffusion method, following the procedures of the Clinical and Laboratory Standards Institute (CLSI). A standard panel of antimicrobials including amikacin (30 µg), amoxicillin + clavulanic acid (20/10 µg), ampicillin (10 µg), cefpodoxime (10 µg), cephazolin (30 µg), cloramphenicol (30 µg), enrofloxacin (5 µg), gentamicin (10 µg), kanamycin (30 µg), nitrofurantoin (300 µg), tetracyclin (30 µg), tilmicosin (15 µg) and trimethoprim+sulphametoxazole (1.25/23.75µg) was tested against *E.coli* isolates. The strains were classified as resistant, susceptible or intermediate to the tested antimicrobials by interpreting the zones of growth inhibition according to the CLSI. Intermediate strains were grouped with the resistant ones. The *E.coli* strains included in this study showed the following percentages of resistance: tilmicosin (87.2%), tetracyclin (73.6%), ampicillin (70.7%), cephazolin (57.7%), amoxicillin + clavulanic acid (43%), enrofloxacin (34.8%), trimethoprim+sulphametoxazole (29.9%), cefpodoxime (28.7%), kanamycin (23%), gentamicin (13%), cloramphenicol (10.6%), nitrofurantoin (6.3%), amikacin (2.9%). Empirical antimicrobial therapy is often instituted while awaiting for the results of culture and sensitivity testing. The sensitivity testing results obtained in this study showed that the antibiotics classified as highest priority critically important antimicrobials (3rd and 4th generation cephalosporins, quinolones, macrolides and polymyxins) by WHO (3) do not necessarily represent the best choice in terms of in vitro efficacy and some therapeutic alternatives, based on less critical antimicrobials are to be preferred. Periodic monitoring of pathogens isolated from UTI and their susceptibility patterns are helpful in guiding the first line empirical therapy and can also be effective for monitoring the eventual spread of resistant bacteria.

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Vespa

Surveillance of antimicrobial usage in livestock of Thailand: a model of drug supply chain.

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Abstract

WHO global action plan has lunched guideline to optimize use of antimicrobial agents in human and animal. Patterns of antimicrobial use in livestock are widely different in specie level. Pig production seems to consume more antibiotics than other species. The current study will approach on surveillance of antimicrobial consumption in animal, focusing on food animal production. To establish antimicrobial usage (AMU) monitoring system, setting a pilot AMU model in high density farming area is planned. Data collection is limited to agents in ATC-Vet of QJ01 group. Total amount of annual sale data of 28 antimicrobial veterinary drugs consumption in animal is presented. Amoxicillin, tiamulin, chlortetracycline and tylosin are most common antibiotic used in pig farms. The preliminary result is providing number of antibiotic used in farm including systemic review of stakeholder mapping, antimicrobial agents supply chain.

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Antibacterial activity of semi-purified compound from pyroligneous acid of oil palm shell against infected bacteria associated with wound healing in animal.

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Abstract

The increasing prevalence of microbial infections associated with wound healing in animal, caused by bacteria, are a serious health problem in animal. The new antimicrobial against from natural product has been wildly developed. Pyroligneous acid also called wood vinegar is brown-red transparent liquid as a by-product during pyrolysis to manufacture biomass charcoal. Pyroligneous acid prepared oil palm kernel shell has been a value-added product of biomass waste from oil palm industry in Southeast-Asia. The purpose of this study was to evaluate the antibacterial activity of semi-purified compound form pyroligneous acid of oil palm shell prepared by carbonization against important three pathogenic bacterial in animal including Staphylococcus aureus, Escherichia coli andPseudomonas aeruginosa. The resulted revealed that semi-purified compound was found to be effective against the investigated pathogens. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) on S. aureus and P. aeruginosa were 15.62 ug/ml and 31.25 ug/ml, respectively, while E. coli was inhibited and killed with concentration of 31.25 ug/ml and 62.50 ug/ml, respectively. However, the MICs of tetracycline against three bacterial stains range from 0.48 to 0.96 ug/ml. Time kill determination indicated that semi-purified compound form wood vinegar of oil palm shell at the concentration of 4 xMIC had rapid killing effect in all tested bacteria and eliminated all bacteria within 4 hours. However, efficacy of the compound was still lower than that of tetracycline at the concentration of 2 xMIC. The compounds and their concentrations in semi-purified sample were indicated by gas chromatography/fame ionize detector, which found that phenol and its derivative were major components with summary concentration of 1,013.54 mg/ml. Overall could be concluded that extracted compound isolated from wood vinegar of oil palm shell has potential to use as alternative antipathogenic bacteria against supported wound healing therapy in animal.

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DIPARTIMENTO DI SCIENZE VETERINARIE PER LA SALUTE, LA PRODUZIONE ANIMALE E LA SICUREZZA ALIMENTARE

Reducing the spread of antimicrobial resistance in animal farms for the health protection of workers.

Paola Tomao[1,*], Lebana Bonfanti[2], Marta Bottino[3], Giorgio Franceschini[3], Elisa Martello[3], Ilary Millet[3], Francesca Zaltron[4], Anna Rosa Favretto[4], Nicoletta Vonesch[1], Alessandro Mannelli[3]

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Abstract

The emergence of antimicrobial resistance (AMR) is a complex problem driven by many interconnected factors regarding human, animal and environmental health sectors. An interdisciplinary effort within the One Health approach is necessary to face the phenomenon, as further evidenced by the Council conclusions of the 23 July 2016. In the occupational field, farmers are at risk of acquiring from animals and farm environment AMR agents. The aim of the present study, which represent the first part of a project funded by the National Institute for Insurance against Accidents at Work (INAIL) and focused on prevention purposes, is to identify farming practices associated to the risk of exposure to AMR. We adopted a mixed methods research design, integrating qualitative and quantitative techniques. By using fattening turkey production as a case study, we reconstructed farming phases, as well as corresponding working practices, based upon semi-structured interviews of public and private veterinarians and farmers in Northern Italy. Working practices were classified and ordered in terms of the probability of exposure of farmers to AMR, by a modified FMEA (Failure Modes and Effect Analysis), based upon information on prevalence of agents in animals and in the farming environment, and on AMR transmission routes. Scientific evidence to estimate prevalence of AMR in poultry farming and associations between working practices and the transmission to farmers was obtained by a systematic literature review. Preliminary results showed that vaccination of turkeys was characterized by the highest risk of exposure to AMR, due to direct and repeated contact with animals, by workers without personal safety devices. Removal and milling of litter ranked second in the risk prioritization, given the exposure of farmers to high dust level. Appropriate risk communication and risk mitigation measures will be produced, and communication of preventive measures to farmers and other stakeholders will be implemented by means of a website.

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Antifungal activity of dual-combined essential oils on *candida albicans* isolated from cattle.

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Abstract

The antifungal effect of 3 selected essential oils (dually combined) was tested against Candida albicans yeasts by use of microdilution broth method (M27-A2, CLSI, 2009). Essential oils (EOs) of clove (Syzygium aromaticum), cinnamon (Cinnamomum zeylanicum) and thyme (Thymus vulgaris) were tested in mutual dual combinations at concentration from 0.64% to 0.01%. The validity of testing was confirmed on the basis of reference strain (Candida parapsilosis ATCC 22019) sensitivity to fluconazole. Samples of Candida albicans (n=15) were isolated from swabs collected from predilection areas of cattle (perineum, external auditory canal). Pure Candida isolates were cultivated on Sabouraud´s dextrose agar with chloramphenicol at 35°C for 24 hours and then suspended to 103 CFU/ml in 0.9% saline solution. The standardized suspension was used immediately. The binary dilution of EOs to required concentration was conducted on 96-well microdilution plate and in test tubes (in 3 combinations: clove+cinnamon, clove+thyme and cinnamon+thyme). EOs were applied on the microdilution plate horizontally and vertically with decreasing concentration from right to left and from top to bottom according to the methodology used. Suspension of Candida albicans was applied to each well containing mixture of 2 EOs, one column of microplate served as negative (without Candida) and one as positive (without EOs) control. After 24-hour incubation at 35°C, the inhibitory concentrations were read in compliance with the checkerboard test. Effect of EOs combination was evaluated according to the formula FICI=FIC1/MIC1+FIC2/MIC2, where FICI is the fractional inhibitory concentration index, FIC1 and FIC2 are MICs of EOs combined and MIC1 and MIC2 are MICs of EOs alone. The results were interpreted as follows: FICI≤0.5 synergistic, >0.5<2 additive, ≥2<4 indifferent and FICI>4 antagonistic. Our results show the additive effect of each three EOs combinations. MIC of clove alone (MIC1) was 0.12±0.07% and MIC of cinnamon alone (MIC2) 0.07±0.02%, FICI of their combination was 0.5±0.1, which corresponds to additive effect. Similar additive effect was observed by combination of clove and thyme (MIC1=0.07±0.05%, MIC2=0.06±0.02%, FICI=0.8±0.2) and by combination of cinnamon and thyme (MIC1=0.08±0.02%, MIC2=0.04±0.01%, FICI=0.625±0.15). These results indicate that appropriately combined EOs can increase their antifungal potency. This work was supported by the Slovak Research and Development Agency under the contract No. APVV-15-0377.

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Molecular identification of antimicrobial resistance (AMR) genes in cow raw milk.

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Abstract

Raw milk is rich in bacteria and can be a source of antimicrobial resistance (AMR), depending on the presence and abundance of antimicrobial-resistant genes among the bacteria in the farm and animal environment. There is a strong evidence that human consumption of milk carrying antibiotic-resistant bacteria has resulted in the transfer of these bacteria to consumers and infections by pathogens with the same resistance profile. We describe the application of culture-indipendent techniques used to I) identify the presence of tetracycline resistance genes (tet) in bovine raw milk by a set of multiplex PCRs; II) evaluate the presence of other antibiotic resistance genes by Next Generation Sequencing (NGS) analysis. Specific primer pairs were used for PCR amplification of 14 tetracycline resistance genes commonly found in Gram-positive and Gram-negative bacteria. Combinations of primers were used to detect 4 specific groups of tet genes: (I) tet(B), tet(C), tet(D); (II) tet(A), tet(E), tet(G); (III) tet(K), tet(L), tet(M), tet(O), tet(S); (IV) tetA(P), tet(Q), tet(X). Totally, 23 raw milk samples collected from farms located in Piemonte and Liguria regions, were analyzed by multiplex PCRs. Afterwards, NGS method on Illumina Miseq was directly used on tet-positive samples. The results of multiplex PCRs showed that 56.5% (13/23) milk samples were positive for at least 1 of the tet genes: tet(G) n=3, tet(K) n=1, tet(L) n=4, tet(O) n=5, tet(S) n=4 and tet(M) n=3. Of these samples, 46% (6/13) presented different combinations of resistance genes: 15% tet(L)+tet(O)+tet(S), 15% tet(L)+tet(O), 8% tet(K)+tet(M), 8% tet(O)+tet(S). NGS analysis confirmed the presence of tet genes in all 13 samples and allowed to detect a number of other resistance genes: strA (streptomycin), bla/cfx (beta-lactams), aph/ant/aad (aminoglycosides), dfr (trimethoprim) and flo (chloramphenicol). The findings of our study indicate that milk can be a reservoir of bacteria carrying resistance genes with a potential for spreading through the food chain. When setting multiplex PCRs, tetracycline was selected as a model because resistance against this antibiotic has been extensively documented among food-borne bacteria. Furthermore, the results highlight that the use of culture-independent methods are useful to provide precise information on AMR situation in the territory along the production chain.

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Multiresistant *Mannheimia haemolytica* isolates as a cause of therapy failure for bronchopneumonia in beef farms.

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Abstract

Mannheimia haemolytica is considered as the predominant bacterial pathogen associated with infectious bronchopneumonia, an economic and animal welfare very important disease in cattle production systems worldwide. Infectious bronchopneumonia is the main indication for antimicrobial use in calves. Therefore it receives considerable attention in countries where current antimicrobial use in food animals is questioned. With exception of the veal industry, available reports on respiratory pathogens in beef cattle show relatively low resistance levels. This study describes the isolation of multiresistant *M. haemolytica* isolates in beef cattle suffering from infectious bronchopneumonia. From February 2016 to February 2018, 11 multiresistant *M. haemolytica* isolates were obtained from 11 different beef herds in Belgium. All animals showed clinical signs of infectious bronchopneumonia and lung consolidation on thoracic ultrasonography. Broncho-alveolar lavage (BAL) samples were taken to identify the causative pathogen(s). BAL samples were cultured on Columbia blood agar and isolates were identified by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS). Susceptibility testing for 11 commonly used antimicrobials was performed with Minimal Inhibitory Concentration (MIC)-gradient strip test (ampicillin, tetracycline, penicillin, florfenicol, enrofloxacin, trimethoprim-sulfonamides, amoxicillin-clavulanic acid, doxycycline) and disk diffusion (ceftiofur, tylosin, tulathromycin). Quality control strains (*E. coli* ATCC 259222, *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212) were included in the study. In all cases *M. haemolytica* was abundantly present. All isolates were resistant for a minimum of 6 antimicrobials. Antimicrobial resistance was seen of 7, 8, 9 and 10 antimicrobials in 18%, 9%, 45% and 9% of the isolates, respectively. All isolates might be susceptible for amoxicillin-clavulanic acid (MIC-values ≤ 2µg/mL) and resistant for doxycycline (MIC-values ≥ 8µg/mL), although no clinical breakpoints for latter antimicrobials are available. These findings show the presence of multiple multiresistant, clinically relevant *M. haemolytica* isolates in beef herds in Belgium, resembling recently reported multiresistant isolates from France and Germany. These isolates can cause inappropriate antimicrobial therapy when the first line antimicrobials are used in these cases, stressing the importance of sampling at an early stage of the outbreak.

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Vespa

Antimicrobial susceptibility of *Mycoplasma synoviae* isolates from outbreaks in Dutch poultry 2001-2018.

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Abstract

To further reduce and refine the use of antimicrobial drugs in livestock, monitoring of antimicrobial resistance (AMR) of veterinary pathogens is of utmost importance. Therefore, a project is running with the ultimate aim to obtain a nationwide, representative and reliable system for monitoring of AMR in pathogens from livestock, dogs, cats and horses in the Netherlands. As part of this project, the feasibility of a system for active monitoring of AMR of *Mannheimia haemolytica* (MHA) and *Mycoplasma* spp. (MYC) in veal calves was investigated. Veal calf veterinarians were requested to actively submit nasal swabs from veal calves meeting the inclusion criteria of the study (like 2-8 weeks of age and clinical signs pointing to respiratory disease). In total 3-5 calves per farm, originating from 35 different farms were sampled. Samples were submitted to GD Animal Health (GD AH) for bacteriological examination and AMR testing by broth microdilution. In addition, GD AH routinely conducts a 'passive' monitoring of AMR: veal calves are submitted for post-mortem examination to identify the cause of death. In case of relevant pathological findings, samples from – for example – the respiratory tract are collected and submitted for bacteriological examination and AMR testing. The data on AMR of MHA and MYC available via this passive monitoring by GD AH were also collected and compared to the actively obtained AMR results. All results were evaluated for representativeness regarding farm size and geographical location. A high response in actively submitted nasal swabs was observed. Furthermore, it was found that at least five nasal swabs from five different calves per farm should be submitted to obtain one MHA isolate per farm. For MYC, the applied sampling strategy (3-5 calves/farm) resulted in at least one MYC isolate per farm in all farms except one. Comparing results from actively and passively obtained samples, it was concluded that for monitoring of AMR of MHA, results of both systems can be combined. For MYC however, it should be reconsidered whether passively obtained results for AMR have an added value over the AMR results from actively obtained samples as the active and passive AMR results differed.

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Antimicrobial resistance in selected respiratory pathogens of veal calves: a pilot study towards a nationwide representative antimicrobial resistance monitoring system in livestock.

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Abstract

Mycoplasma synoviae (M.s.) is a pathogenic mycoplasma with great economic impact in commercial poultry due to disease problems. In cases with severe clinical signs antibiotic treatment is given to reduce the impact of disease in the flock. Several antimicrobials are registered for treatment of M.s. infections. To allow more prudent use of antimicrobials, information on antimicrobial susceptibility profiles of M.s. is important. Recent publications on antimicrobial susceptibility (AMS) of M.s. are scarce. This study was performed to examine the AMS of M.s. isolates obtained from Dutch poultry in the years 2001 to 2018. The collection consists of 48 M.s. isolates from commercial and backyard poultry (chickens and turkeys) from the Netherlands, obtained between 2001 and 2018. The identity of M.s. isolates was confirmed by PCR. Minimal inhibitory concentrations (MICs) were determined by broth microdilution using commercially available MIC plates. Mycoplasma Experience (ME) broth was used as growth medium, and a standard dilution of culture was added to each well so that 0.5x10e3–10e5 CCU per ml were delivered in a final 50 ml volume. Plates were sealed, incubated under aerobic conditions at 37°C, and after seven days minimal inhibitory concentrations (MICs) were read. The in vitro susceptibility to four different antimicrobials was examined. For each antimicrobial agent the range of MIC-values, the MIC50- and MIC90-values were calculated. Most of the susceptibilities were determined as lower as or equal to the lowest concentration tested or higher than the highest concentration tested, except for oxytetracycline. Results show lowest MIC values for tylosin (MIC50 of ≤0.5 and MIC90 of ≤0.5 µg/mL, respectively), tilmicosin (MIC50 and MIC90 both being ≤4 µg/mL), and tiamulin (MIC50 and MIC90 both being ≤0.5 µg/mL). Susceptibility for oxytetracycline comprised more MIC-values (MIC50 of 4 µg/mL and MIC90 of 8 µg/mL, respectively). Results show good susceptibility to the antimicrobials tested, except for oxytetracycline. Further research into the specific genes for tetracycline resistance is recommended.There is a great need for the development of a standardized and harmonized method for M.s.-specific AMS testing, which also may contribute to establish specific clinical breakpoints. Therefore, currently it is not possible to interpret the MIC-values into treatment advises for the field.

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Use of antimicrobials and its association wit biosecurity in nine Finnish farrow-to-finish pig herds.

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Abstract

The use of antimicrobials in pig production is relatively low in Finland compared with other European countries, because it is only recommended for therapeutic purposes. However, since the pigs are often treated based on the diagnosis of herd personnel, it is doubtful whether antimicrobials are being used prudently. The present study therefore investigates antimicrobial usage (AMU) in the Finnish pig herds, and its association with the biosecurity status of the herds, in order to identify best practices for the prudent use of antimicrobials. In total, nine farrow-to-finish pig herds in western Finland were included in the study. The number of animals were collected through the Finnish Swine Registry system. Data for the use of antimicrobials for one year before the first herd visit (1Y) were collected from the National Health Classification Registry program that provides dates and indications for treatments and types and dosages of antimicrobials used for different age groups. AMU was calculated as the amount (mg) of active substance used per population correction unit (kg) in different age categories during 1Y. The biosecurity status of the study herds were scored using the Biocheck. UGentTM. The AMU for suckling piglets was higher than those for fatteners (55 mg/kg ± 111 vs. 6 ± 5, $P < 0.05$), and tended to be higher than those for weaners or breeders (vs. 14 ± 24 or 17 ± 18, $P < 0.10$, for both). Of the total six active ingredients, Penicillin was the most commonly (65 %) used on the nine study herds, and it was most frequently used for suckling piglets during this 1Y period. AMU for weaners was correlated with internal biosecurity sub-categories, i.e. 'cleaning and disinfection' ($n = 9$, $r2 = 0.71$, $P < 0.05$). Consequently, suckling piglets is the age group with the biggest AMU compared with the other age groups in the study herds where antimicrobials are used for therapeutic purpose. It can be speculated that the need for the treatment of the pigs might be increased in the herds, if the farmers have had more opportunities to observe the health status of the pigs. However, further study will be needed to demonstrate the causal relationship between antimicrobial usage and farmers` attitude.

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