“Antimicrobial resistance:

a challenge for public health, animal health and environment”

Scientific symposium

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Brussels – webinar

Program

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Keynote Presentation: Antimicrobial stewardship in companion animals: why, what and how?

Els Broens – Utrecht University

The increase of antimicrobial resistance (AMR) is recognized as a threat for modern medicine and public health. To help control AMR, responsible antimicrobial use (AMU) is warranted and a decrease in inappropriate AMU is necessary, both in human and veterinary medicine. In human healthcare, successful antimicrobial stewardship programs (ASPs) have been implemented worldwide to improve appropriate AMU. In veterinary medicine, ASPs are rather new and therapeutic guidelines and compliance to these guidelines are less established compared to human medicine. Initially, the focus to reduce AMU was mostly on food producing animals and a combination of compulsory and voluntary action resulted in a significant reduction in AMU in many EU countries. More recently, focus has broadened to companion animals as most action plans and (inter)national legislation are applicable to all animal species. The relevance of a prudent use of antimicrobials (AMs) in companion animals is demonstrated by increasing resistance rates in these animals and their owners. The direct and close contact between pets and humans facilitates transfer of microorganisms, including resistant bacteria. Although total AMU in companion animals overall is relatively low compared to food producing animals, the majority of antimicrobials used in companion animals belong to the critically important antimicrobials for human medicine according to the World Health Organization (WHO). Several studies reveal that critically important AMs are extensively used in companion animals and that AMs are prescribed far more often than recommended. The largest room for improvement regarding AMU in companion animals lies within the quality of use, not so much in the quantity. To optimize AM prescribing quality, proper insight in rational and appropriate AM prescribing is needed. This is a complicated process as the quantity of AMU alone is not sufficient. Insight in AM prescribing quality requires linking prescribing to clinical indications and analysis whether the prescribing decision is according to guidelines, first with respect to prescribing an AM yes/no, and second, with respect to the choice and the duration of AM treatment. This information is often not available from easily available data, but essential in setting optimal targets and providing clinicians with informative feedback highlighting targets for improvements and change. Several studies and pilots on ASPs in companion animals have been performed in the last decade with varying degrees of success. The biggest challenge for the future is to join forces with other animal sectors, with the human sector and many other stakeholders to develop ASPs that are applicable and financially feasible for most companion animal clinics.

First Belgian Report of Ertapenem Resistance in an ST11 *Klebsiella pneumoniae* Strain Isolated from a Dog Carrying bla_{SCO-1} and bla_{DHA-1} Combined with Permeability Defects

Hanne Debergh [1,3,5], Margo Maex [2], Cristina Garcia Graells [1], Cécile Boland [3], Marc Saulmont [4], Koenraad Van Hoorde [1] and Claude Saegerman [5]

[1] Service Foodborne pathogens, Sciensano, B-1050 Brussels, Belgium, Hanne.Debergh@sciensano.be (H.D.), MariaCristina.GarciaGraells@sciensano.be (M.C.G.G.), Koenraad.VanHoorde@sciensano.be (K.V.H.)

[2] Service Bacterial diseases, Sciensano, B-1050 Brussels, Belgium, Margo.Maex@sciensano.be

[3] Veterinary bacteriology service, Sciensano, B-1050 Brussels, Belgium, Cecile.Boland@sciensano.be

[4] Association Régionale de Santé et d'Identification Animales, B-5590 Ciney, Belgium, marc.saulmont@arsia.be

[5] Research Unit in Epidemiology and Risk analysis applied to Veterinary sciences (UREAR-ULiège), Fundamental and Applied Research for Animal and Health (FARAH) Center, University of Liège, B-4000 Liège, Belgium, Claude.Saegerman@uliege.be

Klebsiella pneumoniae of sequence type (ST) 11 is a hyper-epidemic nosocomial clone spreading worldwide among humans and emerging in pets. This is the first report, to the best of our knowledge, of an MDR *K. pneumoniae* ST11 carrying bla_{SCO-1} and bla_{DHA-1} isolated from a companion animal in Belgium. Antimicrobial susceptibility testing (AST) of the isolate performed by broth microdilution following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines revealed resistance to eight different classes of antimicrobials including carbapenems, in particular ertapenem, third-generation cephalosporins and fluoroquinolones. A hybrid approach, combining long and short-read sequencing, was employed for *in silico* plasmid characterization, multi-locus sequence typing (MLST) and the identification and localization of antimicrobial resistance (AMR) and virulence-associated genes. Three plasmids were reconstructed from the whole-genome sequence (WGS) data: the conjugative IncFIB, the non-mobilizable IncR and the mobilizable but unconjugative ColRNAI. The IncFIB(K) plasmid carried the bla_{SCO-1} gene, while IncR carried bla_{DHA-1}, both alongside several other antimicrobial resistance genes (ARGs). No virulence genes could be detected. Here, we put forward that the resistance to ertapenem, associated with susceptibility to imipenem and meropenem in *K. pneumoniae*, could be related to the presence of bla_{SCO-1} and bla_{DHA-1} combined with permeability defects caused by point mutations in an outer membrane porin (*OmpK37*). The presence of the bla_{SCO-1} gene on a conjugative IncFIB(K) plasmid is worrisome as it can increase the risk of transmission to humans, to animals and to the environment.

Can improved farm biosecurity reduce the need for antimicrobials in food animals? A Scoping Review

Pankaj Dhaka[1,2*], Ilias Chantziaras[1*], Deepthi Vijay[3], Jasbir Singh Bedi[2], Iryna Makovska[1], **Evelien Biebaut[1]** and Jeroen Dewulf[1]

[1] Faculty of Veterinary Medicine, Department of Internal Medicine, Reproduction and Population Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium
[2] Centre for One Health, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana 141004, India
[3] Department of Veterinary Public Health, College of Veterinary and Animal Sciences, Mannuthy, Thrissur 680651, India

Introduction

Limited and judicious antimicrobial usage (AMU) is considered as the key to save the success of human and veterinary medicine in treating infections. With the limited alternatives for antimicrobials, farm biosecurity (and herd management) is considered a promising tool to mitigate the non-judicious AMU and to maintain animal health, production, and welfare. The present scoping review aims to analyse the effect of farm biosecurity on AMU in livestock systems and formulate recommendations.

Materials and methods

Peer-reviewed manuscripts published between 2001-2022 were analyzed by means of the PRISMA framework using PubMed, Scopus and Science Direct databases. After applying the inclusion criteria, 27 studies were found to assess the effect of farm biosecurity (or management practices) on AMU at the herd/farm level in quantitative/semi-quantitative terms.

Results and conclusion

These studies were carried out in 16 countries, of which 74.1% (20/27) were from 11 European countries. The highest number of studies were from pig farms [51.8% (14/27)], followed by poultry (chicken) farms [25.9% (7/27)], cattle farms [11.1% (3/27)], and a single study from a turkey farm. Two studies include both pig and poultry farms. Most of the studies were cross-sectional [70.4% (19/27)], seven were longitudinal and one was a case-control study. Complex interactions were observed among various factors influencing AMU, such as biosecurity measures, farm characteristics, farmers' attitudes, availability of animal health services, stewardship, etc. A positive association between farm biosecurity and reduction in AMU was observed in 51.8% (14/27) of the studies and 18.5% (5/27) showed that improvement in farm management practices was associated with a reduction in AMU. Two studies highlighted that coaching and awareness among farmers may lead to a reduction in AMU, and a single study on economic assessment concluded biosecurity practices as a cost-effective way to reduce AMU. On the other hand, five studies showed an uncertain or spurious association (due to confounders like a recent outbreak) between farm biosecurity and AMU. We recommend for the reinforcement of the concept farm biosecurity, especially in lower- and middle- income countries. Further, there is need to strengthen the evidence on the association between farm biosecurity and AMU in region- and species-specific farm settings.

Linezolid-resistant isolates in food-producing animals in Belgium

Michaël Timmermans[1,2], Bert Bogaerts[3], Kevin Vanneste[3], Sigrid C. J. De Keersmaecker[3], Nancy H. C. Roosens[3], Carole Kowalewicz[1], Guillaume Simon[1], Maria A. Argudin[4], Ariane Deplano[4,5], Marie Hallin[4,5,6], Pierre Wattiau[1], David Fretin[1], Olivier Denis[6,7] and **Cécile Boland[1]**

[1]Veterinary Bacteriology, Sciensano, Ixelles, Belgium; [2]Faculté de Médecine, Université Libre de Bruxelles, Brussels, Belgium; [3]Transversal Activities in Applied Genomics, Sciensano, Ixelles, Belgium; [4]National Reference Centre-Staphylococcus aureus, Department of Microbiology, Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium; [5]Department of Microbiology, LHUB-ULB, Université Libre de Bruxelles, Brussels, Belgium; [6]Ecole de Santé Publique, Université Libre de Bruxelles, Brussels, Belgium; [7]Laboratory of Clinical Microbiology, National Reference Center for Monitoring Antimicrobial Resistance in Gram-Negative Bacteria, CHU UCL Namur, Yvoir, Belgium

Background: Linezolid is a critically important antibiotic used to treat human infections caused by MRSA and VRE. While linezolid is not licensed for food-producing animals, linezolid-resistant (LR) isolates have been reported in European countries, including Belgium.

Objectives: To: (i) assess LR occurrence in staphylococci and enterococci isolated from different Belgian food-producing animals in 2019 through selective monitoring; and (ii) investigate the genomes and relatedness of these isolates.

Methods: Faecal samples (n = 1325) and nasal swab samples (n = 148) were analysed with a protocol designed to select LR bacteria, including a 44–48 h incubation period. The presence of LR chromosomal mutations, transferable LR genes and their genetic organizations and other resistance genes, as well as LR isolate relatedness (from this study and the NCBI database) were assessed through WGS.

Results: The LR rate differed widely between animal host species, with the highest rates occurring in nasal samples from pigs and sows (25.7% and 20.5%, respectively) and faecal samples from veal calves (16.4%). WGS results showed that LR determinants are present in a large diversity of isolates circulating in the agricultural sector, with some isolates closely related to human isolates, posing a human health risk.

Conclusions: LR dedicated monitoring with WGS analysis could help to better understand the spread of LR. Cross-selection of LR transferable genes through other antibiotic use should be considered in future action plans aimed at combatting antimicrobial resistance and in future objectives for the rational use of antibiotics in a One Health perspective.

Fluoroquinolone resistance in *E. coli* in the broiler production chain: the role of parent stock and hatchery

Moniek Ringenier[1], Jeroen Dewulf[1], Sigrid C.J. De Keersmaecker[3], Kevin Vanneste[1], Bert Bogaerts[3], Junjia He[1], Mathias Devreese[2] and Filip Boyen[2]

[1]Department of Internal Medicine, Reproduction and population medicine, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium;
[2]Department of Pathobiology, Pharmacology and Zoological Medicine, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium;
[3]Transversal activities in Applied Genomics, Sciensano, Brussels, Belgium

Introduction

Although restrictions on the use of fluoroquinolones in livestock have been imposed in Belgium since 2016, high levels of fluoroquinolone (FQ) resistance are still found in commensal *Escherichia coli (E. coli)* in broilers. The purpose of this study was to describe the spread of FQ resistant *E. coli* throughout the broiler production chain from parent stock farms up to day-old broiler chicks and to investigate their role in the dynamics of the spread.

Materials and methods

In this study, 4 broiler parent stock farms were included (which were supplying eggs to 3 different hatcheries). On these parent stock farms, 30 faeces samples, 30 eggs, swabs of the egg trays and information on antimicrobial usage, were collected. The batch of on-farm sampled eggs of each parent stock farm was traced to the hatchery and again 30 eggs of the same batch were sampled on day 18 of hatching. On the day of hatching, 30 day-old chicks were collected from the same batch as well. Environmental swabs were collected in the hatchery from several crucial points where the eggs or day-old chickens came into contact with the environment, being the hatching crates, suction cups, collection belt, and the transport crates of the day-old chicks. Both the outside of the eggs, the crushed eggshell, and the contents of the eggs were analysed following the method of Mehzoud *et al.* (2016) [1]. In all samples, the presence of *E. coli* and FQ resistant *E. coli* isolates was detected by plating on plain MacConkey agar or supplemented with 0.25 µg/ml enrofloxacin (ECOFF). All isolates were identified using MALDI-TOF MS. For a selection of isolates whole genome sequencing was applied to investigate their phylogenetic relatedness using cgMLST analysis, and to detect resistance determinants.

Results

On all 4 parent stock farms, FQ resistant *E. coli* were detected [56.7-93.3%]. A low number of eggs was positive for *E. coli,* both just after laying [0-13.3%] and after 18 days of incubation [0-3.3%]. Except for one egg after laying, no FQ resistant *E. coli* could be found in or on the eggs. In the day-old chicks sampled at the hatchery, 3 out of 4 batches were positive for FQ resistant *E. coli* [0-90%]. On the day of hatching all environmental swabs from the collection belts were positive for FQ resistant *E. coli* and in 3 out of 4 batches, FQ resistant *E. coli* was discovered on the transport crates of the 1-day-old chicks. The results of the whole genome sequencing will be presented during the conference.

References

[1] H. Mezhoud, I. Chantziaras, M. Iguer-Ouada, N. Moula, A. Garmyn, *et al.* (2016). Avian Pathology, 45:4, 493-500

Prevention of calf respiratory pathologies using a new herbal supplement: a preliminary study

Delhez, P.[1], Pirard, B.[1], **Theron L.[1]**, Lhoest E.[2], Rao A.-S.[1]

[1]RumeXperts [2] Miloa

Introduction

Calf respiratory pathologies such as Bovine Respiratory Disease (BRD) are a leading cause of morbidity and mortality in calves before weaning. BRD is complex and caused by a combination of viral and bacterial pathogens associated with environmental risk factors. The disease is multifactorial, involving some combination of stress or reduced immunity allowing several pathogens to emerge. First-line therapies for respiratory diseases commonly involve antibiotics and anti-inflammatories, but preventive treatments have become increasingly important due to antimicrobial resistance as well as health and animal welfare concerns. In particular, plant formulations represent a prevailing but widely unemployed potential medication as alternative or additional preventive treatment on farms. In this context, the objective of this study was to assess the effect of the administration of a new respiratory preventive herbal supplement on calf health and performances.

Materials and methods

A total of 77 calves in 4 farms from the Walloon region of Belgium were involved in the study until the age of 6 month. A total of 37 calves received a placebo and 40 calves received an herbal supplement administrated in feed in powder form from day 1 after colostrum feeding until 10 days. Performance and health parameters (e.g., weight, growth and inflammatory blood biomarkers, lesions of pulmonary parenchyma) were measured at birth, 2-7 days, 21-28 days, weaning and at 6 months for placebo and treated animals. Disease and curative treatment occurrences were recorded. Generalized linear models were used to assess differences in performance and health parameters between the placebo and the treated groups. The farm and value at birth or day 2-7 (when appropriate) were added as covariates in the model to correct for a potential farm effect and baseline effect.

Results and conclusion

Although no significant difference was observed between placebo and treated calves for all the studied parameters, tendency for decreased number of curative antibiotic and anti-inflammatory treatments (54% vs. 38% of calves with at least 1 treatment for placebo and treated groups, respectively), decreased degree of pathology (22% vs. 15% of calves with severe pathology signs) and lesions of pulmonary parenchyma at day 21-28 (14% vs. 8% of calves with lesions), as well decreased disease prevalence until weaning (66% vs. 56% probability of disease occurrence) was observed for calves receiving the herbal supplement. Results of this preliminary study are promising, and new trials are planned to further investigate this new supplement for the prevention of respiratory pathologies in calves.

Abstracts of poster presentations

Detection of antimicrobial resistant Enterobacterales in dairy production areas of differing zinc concentration

E.Anedda[1,2], G.Madigan[3], D.Morris[2], K.Burgess[1]

[1] Food Safety Department, Teagasc Food Research Centre Ashtown, Dublin, Ireland;
[2] Antimicrobial Resistance and Microbial Ecology Group, National University of Ireland Galway, Galway, Ireland;
[3] Bacteriology/Parasitology Division, Department of Agriculture, Food and the Marine, Backweston Complex, Celbridge, Ireland

Introduction

Antimicrobial resistance (AMR) is a critical public health concern and it is acknowledged that a One Health approach is required to address it effectively. Limited information is available on dissemination AMR in the primary food production environment, where the presence of heavy metals may play a role in promoting AMR gene transmission. In Ireland, where agriculture is of key importance, soils are very well mapped in relation to the levels of heavy metals they contain. This includes zinc, which naturally occurs in low and high concentrations across the country.
Therefore, the objective of this study was to evaluating the presence of AMR Enterobacterales in dairy pasture soil and bovine milk filters on farms in high and low zinc areas across Ireland.

Materials and methods

Samples were collected from two distinct areas across Ireland, with varying zinc concentrations. Enterobacterales were enumerated from soil and bovine milk filter samples and the presence of ESBL-producing Enterobacterales (ESBL-PE), carbapenem resistant Enterobacterales (CRE), and ciprofloxacin resistant Enterobacterales (FQR-E) were assessed on selective agars. Suspect colonies were identified by Maldi-TOF. Antimicrobial susceptibility testing (AST) was performed on confirmed Enterobacterales. Additionally, chemical analysis was conducted on soil samples.

Results and conclusion

A variety of AMR Enterobacterales of differing antimicrobial susceptibility were isolated from both sample types. This study demonstrated that the primary food production environment can harbour AMR Enterobacterales, which might enter the food chain and cause a risk for human health.

In vivo testing of the NeoGiANT extract-based formulations in livestock and aquaculture

Anniek Bus[1], Olivier Oben[1], Jürgen Zentek[2], Nuno Silva[3], Martin Faldyna[4], Roberto Bermudez Pose[5], Claudia Serra[6], Maria Celeiro[5], Marta Lores[5], Tommy van Limbergen[1]

[1] Anitom BV, Pierstraat 122, 2630 Aartselaar, Belgium
[2] Freie Universität Berlin, Germany
[3] Moredun Research Institute, Penicuik, UK
[4] Veterinary Research Institute, Brno, Czech Republic
[5] University of Santiago de Compostela, Spain
[6] CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, Porto, Portugal

Corresponding author: Anniek.bus@anitom.be

Introduction

In light of emerging antibiotic resistance in humans and animals and increasingly limited efficacy of current conventional antibiotic treatments, extra effort is expected from livestock farmers to use as few antibiotics as possible. In addition, alternative and broader approaches to treat infections are urgently needed. In order to fight AMR and support livestock farmers in keeping animals with fewer or no antibiotics, NeoGiANT offers an innovative solution based on the known potent natural antimicrobial and antioxidant activities of grape marc extracts, due to their arsenal of phytochemicals. Feed supplemented with the extract will be produced and fed to cattle, swine, poultry and aquatic animals. The effect of the extract based formulations will be evaluated based on health, performance parameters and disease prevention properties.

Materials and methods

After developing and testing the extract (e.g. for stability), premixes are produced and mixed into the feed, these will be used for the *in vivo* trials. Firstly, dose finding studies will be performed in poultry to evaluate efficacy and tolerance, plus adequate dosages will be determined. Male birds (Cobb 500, n=400) will be equally divided amongst four treatment groups (200 mg/kg polyphenols; 750 mg/kg polyphenols; 1500 mg/kg polyphenols; control). Productive performance (growth, feed intake, feed-to-gain ratio) will be measured throughout the 35-d feeding period. After slaughter, samples will be taken for apparent precaecal and total-tract digestibility and other parameters.

Secondly, safety and zootechnical studies will be performed in cattle, swine, poultry and aquaculture species. For each species four treatment groups (three different dosages + one control group) will be used.

Thirdly, with the most effective dosage, infection studies will be performed in these four animal species. For this challenge, specific pathogens for the particular species will be used. E.g. E. Coli (O157) in cattle, E. Coli (O147 and O149) in swine and Eimeria species in poultry. Health and performance parameters will be compared and fecal samples will be collected daily for microbiome analyses.

Finally, field trials will be performed in swine and poultry farms with known history of enteric problems. Zootechnical performances and health will be compared between supplemented and non-supplemented groups.

Results and conclusion

Since this project is still in an early stage, we cannot present any conclusions yet. However, the extract is developed and the *in vivo* trials will start very shortly. The target products to be developed will be designed to control a large number of diseases of paramount importance in animal production, both in livestock (cattle, swine, poultry) and aquaculture. As a result, NeoGiANT aims to provide effective

alternatives to the main antibiotics used in farmed animals, contributing to the goal of reducing their use. At the same time, the speed of emergence of new antimicrobial resistances (AMR) will be reduced, and existing antimicrobial resistances will be better controlled.

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Emergence of methicillin resistant *Staphylococcal aureus* in companion animals

A. Arthi [1], P.V.Tresamol[1], K.Vinodkumar[1] , K. Sindhu Rajan[1]

[1] Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India – 680651

Introduction

Staphylococci are normal commensals of the skin and mucosa of animals and humans and cause opportunistic infections in immunocompromised individuals. Staphylococcal deep pyoderma involves the dermis and hair follicles and is common in dogs. The emergence of MRSA in household pets is of concern in terms of animal health, and more importantly, the potential for animals to act as sources of infection or colonization of human contacts (Weese *et al*., 2006).

Case history and observations

A seven year old female black Rottweiler was presented to the University Veterinary Hospital, Mannuthy, (India) with a history of chronic pyoderma and fever (103.2° F). Skin lesions were extensive involving lateral and ventral chest and abdomen, limbs, periocular, and perianal regions with generalized alopecia, ulceration, crusting, matting of hair, serous and purulent discharge and scab formation.

Materials and methods

Samples from skin lesions were collected aseptically for bacteriological examination. Molecular analyses (polymerase chain reaction - PCR) was performed with specific primers targeting 16S rRNA gene of *S. aureus* (Amin *et al*., 2011), and a second PCR for *mec*A gene amplification. An antibiogram was performed to identify drug resistance.

Results

Primary inoculation in brain heart infusion agar at 37 °C for 24 hours yielded round, convex and creamy yellow coloured colonies which were identified as *Staphylococcus aureus* based on morphology of colony, Gram's stain appearance and by a positive coagulase test. When subcultured in Mannitol salt agar there was change in colour of media from red to yellow because of acid fermentation products.
Molecular confirmation of the isolate revealed it to be methicillin resistant *S. aureus.* Antibiogram showed sensitivity to cephalexin, clindamycin, amikacin and tetracycline and resistance to amoxicillin clavulanate, enrofloxacin, methicillin, ceftriaxone tazobactam and ceftriaxone. Complete resolution of skin lesions after treatment with cephalexin.

Discussion and conclusion

Methicillin-resistant staphylococci pose major clinical challenges in the treatment of canine bacterial pyoderma (Beck *et al.,* 2012). PCR is more sensitive and reliable to detect MRSA infections rapidly than the conventional MIC assays since the presence of *mec*A gene is considered as confirmation of methicillin resistance in bacteria. Pet animals are increasingly being implicated as sources of community associated MRSA (CAMRSA) to humans. Due to the close proximity to animals, veterinary staff and animal owners can be carriers of MRSA (Loeffleret al., 2005).

Optimizing biosecurity in poultry production using a weighted scoring system - alternative approaches to reduce AMU

Arthi Amalraj[1], Hilde Van Meirhaeghe[2], Nele Caekebeke[3], Ilias Chantziaras[1], Jeroen Dewulf[1]

[1]Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium
[2]Vetworks BV, Aalter, Belgium
[3]Biocheck.Gent B.V, Belgium

Introduction

Metadata including farm characteristics, flock size, production aspects like the stocking density of birds and biosecurity measures present on the farm, helps in identifying risk factors for high AMU at farm level. Biosecurity measurements can draw conclusions for targeted antimicrobial stewardship guidelines for poultry production. Disease prevention in the form of improved biosecurity compliance is one of the most promising ways of reducing antimicrobial usage (AMU). Broiler farms consuming more antimicrobials were found to have lower biosecurity scores, indicating that biosecurity can help in reducing AMU (Caekebeke and co-workers, 2021). The study aimed at building new actions to stimulate infection prevention in the poultry sector. A weighted scoring tool (https://biocheckgent.com/en) was developed and validated to measure biosecurity in breeder, turkey, duck, free-range layer and free-range broiler production in a standardized and reproducible manner.

Materials and methods

Questionnaires were drafted each with (turkey=102, duck=108, breeders=119, free-range broiler=100 and free-range layer=126) questions regarding the implemented biosecurity measures and full points gained only when a measure is applied correctly. On occasion, points will be earned by not performing a certain action (e.g. not sharing equipment with other farms). Answers to questions get converted to scores ranging from 0 (total lack of biosecurity) to 100 (full application of biosecurity). Different measures were prioritized and weighed by a panel of poultry experts on the basis of their relative importance for the prevented transmission. The score per question and per subcategory will be multiplied by a weighting factor. The sum of the weighted scores of the questions within a subcategory will be the subcategory score. The scores for external and internal biosecurity were obtained by taking the weighted averages of all subcategories included. The preliminary scoring exercise was coded numerically into a worksheet programme (Microsoft Excel, 2019) before online implementation.

Results and conclusion

On analysis of the completed surveys (since the launch in mid-2022) the following was observed in duck farms (n=30) (71% vs 77%), breeder farms (n=64) (75% vs 81%), turkey farms (n=86) (67% vs 81%), free-range broiler farms (n=13) (66% vs 83%) and free-range layer farms (n=9) (69% vs 74%). The scores for internal biosecurity were higher than external in all types of production. Ducks have a specific reservoir role in the epidemiology of avian influenza viruses and important efforts are necessary to improve biosecurity in the duck industry. Backyard and free-range flocks in the vicinity of commercial farms are a greater risk for transmission of avian influenza. Biosecurity standards in poultry production is receiving more attention with the reemergence of Highly Pathogenic Avian Influenza (HPAI) viruses in Europe. A poultry biosecurity database is now available for future studies which allows benchmarking and in providing farm-specific advice for improvements.

Long-read shotgun metagenomics as a One Health tool to characterize antimicrobial resistance in food-producing environments

Bloemen Bram[1*], Gand Mathieu[2*], Bogaerts Bert, Marchal Kathleen, FARMED consortium, Roosens Nancy, Vanneste Kevin, De Keersmaecker Sigrid C.J.,[3]

[1]Sciensano; [2]UGhent; [3]One Health European Joint Programme; *equal contribution

Introduction

In the past years, food-producing environments have been identified as an important source of antimicrobial resistance (AMR), resulting in persistence in the food chain and transmission to both animals and humans. However, knowledge gaps related to the exact transmission route of AMR genes remain and therefore an accurate inventory of AMR along the food chain is needed. The detection of pathogens carrying AMR genes is frequently done using targeted methods, often requiring culturing and/or isolation. Open approaches, such as shotgun metagenomics, can directly identify all genetic material in a given sample without *a priori* knowledge, possibly offering efficient, rapid and accurate solutions for pathogen detection and characterization along the food chain. However, before it can be applied, metagenomic approaches should be appropriately developed and validated from sampling and DNA extraction to sequencing and data analysis.

Materials and methods

Nanopore sequencing represents a disruptive innovation for microbiology through the generation of long sequencing reads in real-time on a portable device. Compared to short-read sequencing, this technology allows for better detection and scaffolding of microbial genes to their host chromosomes in complex metagenomics samples, improving taxonomic classification and identification of antimicrobial resistance genes. We are developing and optimizing protocols for sensitive metagenomics, by analyzing various materials collected in food-producing environments such as farms. Using a spiked-in defined microbial community standard composed of different bacterial species in various concentrations and with various AMR genes, we assessed these methods with the specific needs of different applications in mind. Moreover, short- and long-read approaches were compared to each other.

Results and conclusion

With the aim to detect specific bacterial species and link them with their AMR genes, protocols for long-read metagenomic sequencing of fecal samples were developed. First, a commercial DNA extraction kit was optimized to obtain DNA of sufficient quantity and quality for long-read sequencing. The possibility of using this protocol on-site was also evaluated. Second, fecal samples were spiked with a defined microbial community standard, and total DNA was extracted with the optimized method and sequenced on a MinION flowcell. Bioinformatics analyses of the sequencing data allowed to identify the spiked species and to link them to their AMR genes in the same sample, except for low-abundance species. Comparable results were obtained using short-read sequencing, but AMR-species links could not be made.

In conclusion, we delivered a proof-of-concept for simultaneous identification of bacterial species and their AMR genes in metagenomics samples, and demonstrated its added value compared to conventional methods. We showed that long-read sequencing can help to achieve a higher taxonomic resolution, by identifying AMR genes and linking them to their hosts. This technology can help to elucidate AMR transmission and exchange along the food chain microbiome. Furthermore, future studies will explore how this new technology can be fully transferred to a fast, easy and direct use on-site, opening up opportunities for AMR monitoring and diagnostics in food chain environments and beyond.

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An in-house 45-plex array for the detection of antimicrobial resistance genes in Gram-positive bacteria

Carole Kowalewicz, Michael Timmermans, David Fretin, Pierre Wattiau, Cécile Boland

Veterinary Bacteriology, Sciensano, Ixelles, Belgium

Introduction

Identifying antimicrobial resistance (AMR) genes and determining their occurrence in Gram-positive bacteria provide useful data to understand how resistance can be acquired and maintained in these bacteria.

Materials and methods

We describe an in-house bead array targeting AMR genes of Gram-positive bacteria and allowing their rapid detection all at once at a reduced cost. A total of 41 AMR probes were designed to target genes frequently associated with resistance to tetracycline, macrolides, lincosamides, streptogramins, pleuromutilins, phenicols, glycopeptides, aminoglycosides, diaminopyrimidines, oxazolidinones and particularly shared among *Enterococcus* and *Staphylococcus* spp. A collection of 124 enterococci and 62 staphylococci isolated from healthy livestock animals through the official Belgian AMR monitoring (2018–2020) was studied with this array from which a subsample was further investigated by whole-genome sequencing.

Results and conclusion

The array detected AMR genes associated with phenotypic resistance for 93.0% and 89.2% of the individual resistant phenotypes in enterococci and staphylococci, respectively. Although linezolid is not used in veterinary medicine, linezolid-resistant isolates were detected. These were characterized by the presence of *optrA* and *poxtA*, providing cross-resistance to other antibiotics. Rarer, vancomycin resistance was conferred by the *vanA* or by the *vanL* cluster. Numerous resistance genes circulating among *Enterococcus* and *Staphylococcus* spp. were detected by this array allowing rapid screening of a large strain collection at an affordable cost. Our data stress the importance of interpreting AMR with caution and the complementarity of both phenotyping and genotyping methods. This array is now available to assess other One-Health AMR reservoirs.

Prevalence and characterization of extended-spectrum β-lactamaseproducing *Escherichia coli* in freshwaters, hospital effluents and wastewaters in Belgium

L. Crettels[1,2], N. Burlion[1], A. Habets[2], E. Delrée[1], A-F. Mouchette[1], D. Thiry[2]

[1] Department of Microbiology, ISSeP, Scientific Institute of Public Service, Liège, Belgium
[2] Bacteriology, Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, Centre for Fundamental and Applied Research for Animals and Health (FARAH), ULiège

Introduction

Antimicrobial resistance (AR) is recognized by the WHO as one of the greatest threats to global health. Enteric bacteria coming from humans and animals (One Health approach) can be found in aquatic systems and be a vector for the spread of AR into the environment. The purpose of this work was to evaluate the prevalence of extended-spectrum β-lactamase-producing *E. coli* (ESBL-EC) in freshwaters, hospital effluents and wastewaters.

Materials and methods

A total of 24 stations were sampled including 17 freshwaters, three hospital effluents and the entry/output of two wastewater treatment plants (WWTPs) during two sampling campaigns (winter/summer) in the Ourthe watershed in 2021. *E. coli* strains were picked up from Brilliance ESBL agar medium after membrane filtration method. Disk-diffusion assays were performed following the EUCAST's recommendations. All the ESBL-EC isolates were tested for the presence of blaCTX-M-1, blaCTX-M-2 and blaCTX-M-9 gene's group by PCR. A subset of isolates (n=40) were selected for whole genome sequencing.

Results and conclusion

A total of 615 ESBL-EC were isolated. Genes belonging to blaCTX-M-1 and CTX-M-9 groups were detected respectively in 72% and 15% of the isolated strains. No gene of blaCTX-M-2 group was found. *E. coli* serotype O18:H7 with sequence type ST1463 was predominant (n=14). β-lactamase genes identified were *blaCTX-M* (n=21), with *blaCTX-M-15* the most represented (n=15), as well as *blaTEM* (n=6), *blaOXA* (n=9) and *blaSHV* (n=9). One of the most observed concerns was the large number of strains containing carbapenemase genes- *blaKPC-3* (n=19), *blaNDM-1* (n=1) and *blaVIM-1* (n=2) -even in freshwaters. This study suggests that hospital effluents and WWTPs contribute to the dissemination of AR into the environment.

Detection of Acquired Antibiotic Resistance Determinants in the Intestinal Microbiota of Food Producing Animals in Hungary

Balázs Libisch1, Gábor Nagy2, Ágnes Csivincsik2, Tibor Keresztény1,3, Péter Papp1, Ferenc Olasz1, Hedvig Fébel4, Zsuzsanna J. Sándor5, Geertrui Rasschaert6, Ellen Lambrecht6, Marc Heyndrickx6, András Szabó2, Melinda Kovács2, Katalin Posta1.

[1]Agribiotechnology and Precision Breeding for Food Security National Laboratory, Institute of Genetics and Biotechnology, Hungarian University of Agriculture and Life Sciences, 2100 Gödöllő, Hungary.
[2]Agribiotechnology and Precision Breeding for Food Security National Laboratory, Institute of Physiology and Nutrition, Hungarian University of Agriculture and Life Sciences, 7400 Kaposvár, Hungary.
[3]Doctoral School of Biological Sciences, Hungarian University of Agriculture and Life Sciences, 2100 Gödöllő, Hungary.
[4]Agribiotechnology and Precision Breeding for Food Security National Laboratory, Nutrition Physiology Research Group, Institute of Physiology and Nutrition, Hungarian University of Agriculture and Life Sciences, 2053 Herceghalom, Hungary.
[5]Research Centre for Aquaculture and Fisheries (HAKI), Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, 5541 Szarvas, Hungary.
[6]Technology & Food Science Unit, Flanders Research Institute for Agriculture, Fisheries and Food, 9090 Melle, Belgium.

Introduction

In June 2017, the European Union announced a new action plan to combat the spread of antimicrobial resistance (AMR): the European One Health Action Plan against Antimicrobial Resistance. The objectives of this action plan include research to understand the epidemiology of AMR, closing knowledge gaps on AMR in the environment and on how to prevent transmission of AMR between animals, humans, and the environment. The aim of this work was the detection of acquired antibiotic resistance genes (ARGs) in the intestinal microbiota of selected food animal species in Hungary by culture-based and metagenomic approaches, with a One Health perspective.

Materials and methods

Altogether 26 domestic pig and wild boar (*Sus scrofa*), common carp (*Cyprinus carpio*) and broiler chicken (*Gallus gallus domesticus*) intestinal content samples collected in Hungary between 2016 and 2021 were examined. Shotgun metagenomic sequencing of gDNA purified from intestinal content samples was performed on Illumina NextSeq 500 and NovaSeq 6000 platforms (Illumina Inc., San Diego, USA) using 2x150 bp paired-end chemistry by Xenovea Ltd. (Szeged, Hungary) and IMGM Laboratories GmbH (Martinsried, Germany), respectively. Sequencing data were analysed by the ABRicate Galaxy version 1.0.1 tool to detect acquired ARGs. Selected faecal samples were screened for the presence of antibiotic-resistant strains of *E. coli* and other intestinal bacteria. The cultured antibiotic-resistant isolates were characterized by molecular and classical microbiological methods.

Results and conclusion

Overall, 59 acquired ARG types were identified by metagenomic analyses from domestic pig and common carp intestinal content samples. The detected ARG types belonged to the antibiotic classes aminoglycosides (27.1%), tetracyclines (25.4%), β-lactams (16.9%), and others, where *tet*(E), a *bla*OXA-48-like β-lactamase gene, as well as *cphA4, ampS* and *qnrS2* were identified only in the examined carp intestinal sample. Metagenomic sequencing demonstrated *tet*(Q), *tet*(W), *tet*(O) and *mef*(A) genes in the gut microbiota of free-living wild boars. By the culture-based approach *E. coli* strains resistant to tetracycline, ampicillin, streptomycin and trimethoprim/sulfamethoxazole and harbouring *tet*(A) and *aadA* ARGs were isolated from domestic pig faecal samples, while a serotype O23:H16 MDR *E. coli* strain carrying *aac(3)-VIa, aadA1, aph(3'')-Ib, aph(6)-Id, sul1, sul2, blaTEM-1B, blaCMY-2* and *tet*(A) determinants was identified from chicken faeces. In conclusion, by use of an up-to-date metagenomic and culture-based approach a broad variety of acquired antimicrobial resistance determinants were

detected in the intestinal microbiota of food-producing animals in Hungary, including ARGs for two critically important antimicrobial classes, the quinolones and the carbapenems.

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Antibiotic resistance in *E. coli* isolated from surface and ground water in areas with intensive livestock farming.

L. Tuts[1,2], M. Heyndrickx[1,3], I. Becue[1], N. Boon[2], P. De Maesschalck[4], R. Eppinger[4], G. Rasschaert[1]

[1] Flanders Research Institute for Agriculture, Fisheries and Food – Technology and Food Unit;
[2] Ghent University, Faculty of Bioscience Engineering: Center for Microbial Ecology and Technology (CMET);
[3] Ghent University, Faculty of Veterinary Medicine, Department of Pathobiology, Pharmacology and Zoological Medicine;
[4] Flanders Environmental Agency

Introduction

Antibiotic resistance poses a severe threat to treat infectious diseases. The problem is globally still increasing because overuse increases the spread of antibiotic resistance. In the context of « One Health », it is crucial to acknowledge the affiliation between both human and veterinary medicine and the spread of AMR in the environment. Contamination of antibiotic resistance in the aquatic environment may occur via several ways and is, among others, associated with the use of raw manure to fertilize arable lands. This manure application is accompanied by a bacterial load, including antibiotic resistant bacteria, which can end up in surface water through run-off and draining, and in groundwater due to leaching through the soil. When antibiotic resistant bacteria from the environment are taken up by humans (directly through food or indirectly through other contact), antibiotic resistance genes may be transferred to bacteria belonging to human gut flora, including pathogens.

Materials and methods

Antibiotic resistance occurrence in the aquatic environment was investigated by sampling regions with intensive livestock production in Flanders; Manure Action Plan locations were chosen as representatives. This resulted in 50 surface water locations (plus a repetition 6 months later) and 50 ground water sites in the *Yser*-basin in West-Flanders (n = 35) and in a part of the *Meuse*-basin located in Antwerp (n = 15). The sampling of surface water took place during March 2022 and a second time during the period September-November 2022. In Flanders, the spread of manure on the land is mostly restricted in the period September-January, possibly leading to a different antibiotic load during these two samplings. Groundwater was sampled from August till December 2022. *E. coli* was isolated on Rapid *E.* coli 2 for water testing (BioRad) and besides ESBL (Extended Spectrum Beta-lactamase producing) *E. coli* was isolated on Brilliance ESBL (Thermofisher). All isolates were profiled towards antibiotic resistance to a panel of 14 different antibiotics (EUVSEC 3, Thermofisher) by comparing determined minimum inhibitory concentrations with epidemiological cut-off values (EUCAST). For ESBL *E. coli*, additional testing was performed for 10 beta-lactam antibiotics by making use of EUVSEC 2, Thermofisher.

Results and conclusion

Overall resistance (against at least one antibiotic) within generic *E. coli*, retrieved from surface water, varied between 58% (March) and 74% (September-November). There was resistance (March–September till November) against sulfamethoxazole (29%-70%), ampicillin (19%-11%), tetracycline (13%-15%), trimethoprim (13%-15%), ciprofloxacin (8%-4%), chloramphenicol (2%-6%), colistin (0%-4%), nalidixic acid (2%-4%), cefotaxime (2%-2%), ceftazidime (2%-2%) and azithromycin (2%-2%). Resistance was simultaneously detected to 7 different antibiotics at most. ESBL *E. coli* (100% resistance against ampicillin, ceftazidime & cefotaxime), isolated from the same samples, exhibited increased resistance levels (up to 10 different antibiotics) for antibiotics tested with EUVSEC 3 (sulfamethoxazole, trimethoprim, tetracycline, ciprofloxacin, nalidixic acid, azithromycin, gentamycin) and the additional testing with EUVSEC 2 testing made it possible to note resistance against the human clinical antibiotics: cefepime, cefoxitin and ertapenem.

Resistance in groundwater was also high (67%), with sulfamethoxazole resistance (50%) most frequently displayed. Besides, a single occurrence of azithromycin, ciprofloxacin, colistin, cefotaxime and ceftazidime resistance was noticed. No ESBL *E. coli* could be detected.

Trends of Antimicrobial consumption in Belgian hospitals using different metrics : A 2012-2021 longitudinal study including the COVID-19 Era

Lucy Catteau[1,2], Laura Bonacini[1], Moira Kelly[1] and Boudewijn Catry[1]

[1] Department of Epidemiology and public health, Sciensano, Brussels, Belgium; [2] Faculty of Medicine and Pharmacy, Université de Mons (UMons), Belgium; [3] Faculty of Medecine, Université libre de Bruxelles (ULB), Belgium

Introduction

Excessive antimicrobial consumption (AMC) is one of the most important drivers of antimicrobial resistance. The aim of this study was to track trends in AMC in Belgian acute care hospitals between 2012 and 2021 and to assess the impact of the COVID-19 pandemic by analyzing reimbursement data, as used in the Belgian Hospitals - Surveillance of Antimicrobial Consumption (BeH-SAC) System and the European Surveillance of Antimicrobial Consumption Network (ESAC-Net).

Materials and methods

Reimbursement data on AMC, number of admissions as well as the number of patient days were available from the National Institute for Health and Disability Insurance for all Belgian acute care hospitals. AMC data were collected using the Anatomical Therapeutic Chemical (ATC) classification system and analyzed using the Define Daily Doses (DDDs) listed in the ATC/DDD Index for 2022. Hospital AMC was measured as the number of DDDs/1000 patient days and DDDs/1000 admissions. The AMC expressed as the number of DDDs/1000 inhabitants/day (DID) were retrieved from the ESAC-Net surveillance. To assess 10-years trends, linear regressions were performed. The changes in AMC between 2019 and 2020 and between 2020 and 2021 were calculated to evaluate the impact of the COVID-19 pandemic.

Results and conclusion

A statistically significant decrease of 17.6% in the consumption of antibacterials for systemic use (ATC group J01) was observed in Belgian hospitals between 2012 and 2021 when expressed in DIDs. A sharp decline of 11.9% in DIDs was observed between 2019 and 2020 while it remained stable between 2020 and 2021 (1.40 DIDs). However, when expressed in DDDs/1000 patient days, a relative increase of 7.5% for the median J01 consumption is observed in Belgian acute care hospitals between 2012 and 2021 with a remarkable 4.8% rise between 2019 and 2020 followed by a slight decrease between 2020 and 2021 (-2.2%). A significant 10-years descreasing trend (-4.4%) is observed when the hospital AMC is expressed in DDDs/1000 admissions despite an increase of 6.5% noticed between 2019 and 2020. A trend towards less frequent use of WHO Access antibiotics in favor of Watch and Reserve antibiotics was detected.

Consistent with findings in other countries, this study reveals an increasing trend in hospital AMC during the pandemic in Belgian acute care hospitals when expressed in DDDs/1000 patient days. Contrasting results were obtained when different denominators were used highlighting the importance of considering different metrics when evaluating AMC in the hospital sector. The denominators should be relevant to the population under surveillance and represent only hospitalized patients. This will allow the proper capture of variations in AMC to tailor stewardship activities.

Contaminations of AMR genes linked to the presence of genetically modified microorganisms in the food and feed chain

Fraiture Marie-Alice[1], De Keersmaecker Sigrid[1], Roosens Nancy H.C.[1]

[1]Sciensano, Transversal activities in Applied Genomics

Introduction

Genetically modified microorganism (GMM) contaminations, both viable cells and DNA, were reported in commercial fermentation products (e.g., additives, enzymes). GMM, commonly used by the industry to produce such food/feed products, are frequently harbouring antimicrobial resistance (AMR) genes as selection markers. Such GMM contaminations in the food/feed chain have therefore raised public health concerns related to potential horizontal transfer of AMR genes to other microorganisms, including pathogens, present in gut microbiota and the environment. Therefore, in order to guarantee food/feed safety and traceability, tools to control and monitor GMM harbouring AMR genes in commercial fermentation products were developed.

Materials and methods

Using publicly available patents related to genetically modified (GM) bacteria commonly used to produce fermentation products, key genetic sequences frequently found in GMM were identified, including the 16S-23S region specific to the *B. subtilis* group, 3 AMR genes (*cat*, *aadD* and *tet-l*) and the pUB110 shuttle vector. In addition, unnatural associations between the pUB110 shuttle vector and *Bacillus sp.* from currently known GMM were obtained and characterized by either whole-genome sequencing, DNA walking or metagenomics, from contaminated fermentation products. qPCR methods specific to these key GMM targets and unnatural sequence associations were developed to screen and identify GMM carrying AMR genes1. The method performance was assessed as specific and sensitive, complying with the Minimum Performance Requirements for Analytical Methods of GMO Testing of the European Network of GMO Laboratories. In addition, regarding safety concerns related to AMR genes, conventional PCR methods followed by sequencing to evaluate the presence of full-length AMR genes were developed1. These methods were applied on 156 fermentation products collected from the European food/feed market, comprising different brands, forms and sectors.

Results and conclusion

A GMM detection strategy was proposed. First, the potential presence of GMM carrying AMR genes is screened using qPCR methods targeting key genetic sequences frequently found in GM bacteria. These screening markers include a 16S-23S region specific to the *B. subtilis* group, the pUB110 shuttle vector and 3 AMR genes conferring resistance to chloramphenicol (*cat*), kanamycin (*aadD*) or tetracycline (*tet-l*). In addition, given associated public health concerns, the presence of full-length AMR genes detected by qPCR is assessed by conventional PCR followed by Sanger sequencing. In case a positive signal for at least one screening element is observed, the presence of GMM is suspected. To prove it, qPCR methods specific to the 5 currently known GMM are used. To monitor GMM contaminations on the European food/feed market, the developed GMM detection strategy was applied on 156 commercial fermentation products. Among these samples, a contamination with known GMM was observed in 70 samples, including 4 samples with viable GMM isolate carrying AMR genes (*aadD*). In addition, AMR genes (*cat*, *aadD* and *tet-l*) were detected in 40 samples for which the presence of unknown GMM was suspected. Based on these results, a first overview of AMR genes linked to GMM contaminations in the food/feed chain was established.

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1 https://www.sciencedirect.com/science/article/pii/S0956713519304621; https://pubmed.ncbi.nlm.nih.gov/32622259/; https://link.springer.com/article/10.1007/s12161-020-01803-6; https://www.sciencedirect.com/science/article/pii/S0168160520304074?via%3Dihub; https://www.nature.com/articles/s41598-020-63987-5; https://www.sciencedirect.com/science/article/pii/S0168160521002890; https://link.springer.com/article/10.1007/s12161-021-02044-x

Unequal progress towards national antimicrobial consumption targets in the ambulatory care sector in Belgium: A 2012-2021 longitudinal study including the COVID-19 era

Moira Kelly[1], Laura Bonacini[1], Boudewijn Catry[1] and Lucy Catteau[1]

[1] Healthcare-associated infections and antimicrobial resistance, Sciensano

Introduction

Inappropriate antimicrobial consumption (AMC) is a key driving force in the emergence of antimicrobial resistance. As such, national and international targets have been established to identify and reduce inappropriate antimicrobials prescription and consumption. The aim of this study was to assess the progress towards such national and international targets, and analyse the impact of the COVID-19 pandemic on AMC in the ambulatory care sector in Belgium utilising antimicrobial reimbursement data as used in the European Surveillance of Antimicrobial Consumption Network (ESAC-Net).

Materials and methods

Data on the reimbursement of antimicrobials, identified by Anatomical Therapeutic Chemical (ATC) classification, together with the number of inhabitants were provided annually by the National Institute for Health and Disability Insurance. Since 2018, the majority of prescribed fluoroquinolones are no longer reimbursed, therefore reimbursement data were combined with national sales data for this class of molecules. The volume of antimicrobials consumed were converted to Defined Daily Doses (DDD) using the WHO ATC/DDD Index for 2022. AMC targets are described for both the volume consumed, in terms of total DID (DDD per 1000 inhabitants per day) and for prescription quality - considering the ratio of narrow- to broad-spectrum antimicrobials and the proportion of other antimicrobial molecules. These metrics were calculated across a ten year period (2012-2021) to assess trends in antimicrobial consumption.

Results and conclusion

Substantial disruption was seen in primary care settings in 2020 and 2021 due to the COVID-19 pandemic, and this is reflected in the AMC reimbursement data. Considering the total volume of AMC expressed in DID, a 19% reduction was observed from 2019 to 2021, representing considerable progress towards the national target of a 40% reduction from 2019 to 2024. However, these reductions were not uniform across antimicrobial classes and progress is slower in the metrics regarding the prescription quality, that encourage the prescription of more targeted and fewer broad-spectrum antimicrobial treatments. For example, while fluoroquinolone consumption continued to reduce from 2019-2020 (13% reduction from 1.38 to 1.19 DID), it increased as a proportion of total J01 consumption reflecting the disproportionately greater reductions in consumption of other J01 antibacterials in 2020. As such, fluoroquinolone consumption remains above 7% of total J01 AMC despite a 5% national target since 2014. This study highlights the importance of considering quantity and quality metrics in AMC surveillance and the potential requirement for more efforts targeted at improving the quality of antimicrobial prescribing behaviour to achieve Belgian's National Action Plan 2020-2024 goals.

Managing health rather than waiting for the need to treat: probiotics as allies for turkey health

Susanne Kirwan[1], Natasja Smeets[1], Valentine Van Hamme[1], Eric N'Guetta[1]

[1]Kemin Europa NV, Toekomstlaan 42, 2200 Herentals, Belgium

Introduction

Turkey production faces a large challenge in reducing therapeutic antibiotic, since they are highly susceptible to disease and show significant mortality ates during the production cycle. All potential tools (nutrition, genetics, vaccination, others) to help prevent antibiotic use are highly in demand. Probiotics are frequently cited as tools to reduce therapeutic antibiotics but exact data on how much reduction can be achieved are scarce. The present study aimed to test the hypothesis that a specific strain of *Bacillus* sp. ATCC PTA-6737 (PB6) has the potential to reduce therapeutic antibiotic use in commercial turkey farming. The hypothesis was based on a body of scientific research showing the microbiome modulating effects of this specific strain.

A classic research trial cannot answer this question, as management and husbandry in research facilities differ in stocking density, flock size, and the skill of labour; to what is typical for commercial conditions. Smaller flock size, from these, is the most limiting to achieve significant results.

Materials and methods

Over a period of 27 weeks, a commercial poultry integrator applied the *Bacillus* sp. PB6. At the same time, vaccination for ORT (*Ornithobacterium rhinotracheale*) was introduced. The trial period started in December, in cooperation with a commercial turkey integrator in Western Europe. The *Bacillus* sp. PB6 strain was applied across all flocks from hatch till slaughter via the drinking water (3x108 CFU/1000L), on every occasion of wet litter or other indications of intestinal health problems. The decision on how to treat intestinal health was left at the discretion of the three respective veterinarians. Historic data of three previous years (where no probiotic was used), were used as a reference. No further change in housing, husbandry, genetics or management conditions happened concurrently.

Results and conclusion

Compared to the three previous years, a numerical decrease in the number of applications of beta-lactams (-13%) and a statistically significant ($p<0.05$) decrease in colistin treatments (-44%) and the grouped other antibiotics (-51%) was observed. The frequency of diseases requiring treatments decreased in the trial period compared to the previous years. The decrease was significant for enteritis (-38%), colibacillosis (-34%) and ORT (-38%).

This trial confirms the hypothesis that addition of *Bacillus* sp. PB6 has a direct effect on antibiotic use. A confounding effect of the ORT vaccine cannot be ruled out, but it is not likely as ORT is a respiratory disease. The data recording of a typical commercial integrator proved sufficient to assess the research question. Further studies in different regions and species other than turkey are needed to extend these findings.

Antimicrobial resistance characterization of methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* isolated from clinical cases in dogs and cats in Belgium

Suzanne Dewulf[1,2], Noah Tilman[2], Jeroen Dewulf[1], Cécile Boland[2]

[1] Veterinary Epidemiology Unit, University of Gent; 2 Veterinary Bacteriology, Sciensano;

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) have been observed in healthy and diseased dogs and cats. The interaction between humans and pets is increasing over the years and this could imply a potential risk of contamination of humans with these bacteria from infected or colonized animals. In addition, there is a possibility of transfer of resistance genes/bacteria such as *mecA* or *mecC* which code for the production of a modified penicillin binding protein, leading to resistance to β-lactam antibiotics. One of the components of the RT 21/1 PET-AMR 1 research project, in a One Health approach, is to assess this risk and characterize the resistance of MRSA and MRSP isolated from clinical cases in dogs and cats.

Materials and methods

Samples were collected by veterinarians and sent to different laboratories for clinical diagnosis. Once confirmed as MRSA or MRSP, the isolates were sent to Sciensano for further investigation. In this study, the isolated strains were confirmed using a triplex real-time PCR method detecting the *Staphylococcus aureus* specific gene, *nuc*, the presence of the *mecA* gene responsible for methicillin resistance and the *mecC* gene variant. The PCR used for the detection of *mec* genes in MRSA was also used to detect *mec* genes in MRSP. The broth micro-dilution method was used to perform MIC determinations for a panel of molecules selected either for their relevance in human medicine and/or their frequent use in veterinary medicine (Sensititre EUST2 panel).

Results and conclusion

Of the 50 MRSA isolates tested, 26 were from cats and 24 from dogs. All strains were positive for the *nuc* gene, 40 for *mecA* (21 cats and 19 dogs), 1 for *mecC* (dog) and 9 have neither the *mecA* nor the *mecC* gene (5 cats and 4 dogs). To our knowledge, the prevalence of *mecC* in dogs remains largely unknown and studies with larger numbers of samples would be required to verify the incidence of *mecC* in canine clinical isolates. For the 108 MRSP isolates, none of them had the *nuc* gene, as expected. Of these 108 strains, 106 are positive for *mecA* (5 cats and 101 dogs), none have the *mecC* gene, leaving 2 strains (1 cat and 1 dog) without either resistance gene. For the 9 (18%) MRSA strains and the 2 (1.8%) MRSP strains lacking typical MRSA and MRSP resistance genes, their methicillin resistance mechanism is yet unknown and further investigation will be required. For all strains tested by PCR, susceptibility testing is underway and will give us more information on their antibiotic resistance. In the context of a "One Health" approach, it is important to continue research on these zoonotic bacteria in order to safeguard human and animal health.

Genomic characterization of antibiotic resistance in *Campylobacter jejuni* isolates from broilers at slaughter in Sweden, 2017-2021

Wonhee Cha[1], Helena Höök[2], Ásgeir Ástvaldsson[2]

[1]Department of Epidemiology and Disease Control, National Veterinary Institute (SVA), SE-751 89 Uppsala, Sweden; [2]Department of Microbiology, National Veterinary Institute (SVA), SE-75189 Uppsala, Sweden.

Introduction

Campylobacter is the most common bacterial cause of human gastroenteritis in the world, and chicken is the most important source of *Campylobacter* for human infections with 50–80% of human cases attributed to chicken reservoir. In Sweden, *Campylobacter* has been monitored in slaughter batches of broilers since 1991, in which cecal samples from every slaughterbatch are collected for *Campylobacter* culture. Here, we aimed to study the collected isolates further for the antibiotic resistance using whole genome sequencing (WGS).

Materials and methods

A total of 767 *C. jejuni* isolates collected from broilers in 2017-2021 were subjected to WGS using Nextera library kit and Illumina platform. The raw reads were assembled using SPAdes v3.14.0 and analyzed for the presence of genes encoding for antibiotic resistance using AMRFinderplus.

Results and conclusion

A total of 743 (96.9%) among 767 *C. jejuni* isolates from broilers had at least one gene encoding for resistance against antibiotics. Genes for beta-lactam resistance were the most prevalent (95.3%; n=731), followed by quinolone (20.5%; n=157), tetracycline (7.2%; n=55), macrolide (3.9%; n=30) and aminoglycoside (0.4%; n=3). For beta-lactamase, *blaOXA-193* was most prevalent (n=377), followed by *blaOXA-184* (n=87), *blaOXA-461* (n=75), *blaOXA-61* (n=31), *blaOXA-447* (n=30). In total, 28 different *blaOXA* genes were observed. For tetracycline resistance, only *tet(O)* gene was observed, while point mutations detected for quinolone and macrolide was *gyrA*_T86I and *50S*_L22_A103V, respectively. For aminoglycoside, *aph(3')-IIIa* (n=1) and point mutation in *rpsL*_K88R (n=2) was observed. Forty-one isolates (5.3%) had genes encoding resistance for more than 2 antibiotic classes: 28 isolates had beta-lactam, quinolone and tetracycline resistance genes: 11 had beta-lactam, macrolide, and quinolone resistance genes; 1 had beta-lactam, quinolone, and amikacin resistance genes and 1 had macrolide, quinolone and tetracycline resistance genes. There was no clear trend observed over time for the frequency and distribution of isolates with the kind and number of resistance genes. Further analysis is on the way to investigate the association of the findings with phylogenetic lineage and also the distribution within and between farms over the years in relation to the phylogenetic lineage. In addition, the presence of plasmids and their link to the observed resistance genes will be investigated to estimate the impact of horizontal transfer in the spread of antibiotic resistance in the broiler population.