Antibiotic stewardship and the responsible use of macrolides

Antibiotics are needed in animal production to effectively treat infections such as mycoplasmosis. Not being able to treat animals with diseases is unacceptable on the grounds of welfare and it is the general responsibility of the veterinarian to act upon this firm guideline.

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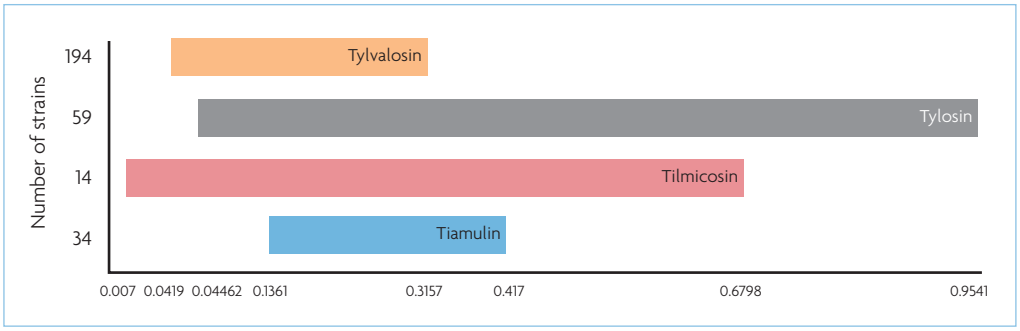

Fig. 1. Ranges of MIC values of different strains analysed together versus the most used antibiotics to treat mycoplasma infection.

To safeguard the future usage of antibiotics within the sector of animal production it is mandatory to understand and guide users towards the principals of antibiotic stewardship. One of the primary reasons behind the establishment of antimicrobial stewardship guidelines is the worrying increase of resistance to antibiotics left to treat animals but also human patients.

Several government bodies and NGOs have published such guidelines towards antimicrobial stewardship.

These include practical advice on how to implement proper antibiotic usage in animal production, such as the attempts made by the EMA-AMEG (European Medicines Agency's working group 'Antimicrobial Advice Ad Hoc Expert Group') guidelines.

To ensure greatest user acceptance and practical application it is imperative that these existing guidelines are transparent and do not contradict each other.

To secure the responsible usage of macrolides in poultry production understanding the resistance situation and using sound treatment advice are a good combination.

What kind of antibiotics are there?

The full complexity of antibiotic selection must be understood to make the correct treatment choice. Treatment choices need to be targeted as no antibiotic drug exists to treat each and every disease in poultry production.

Under the umbrella of antimicrobial stewardship, there are two types of usage considered in animal production: metaphylaxis and therapeutic use in the face of disease.

In this article, the example of mycoplasma in poultry production and the responsible usage of macrolides to treat against mycoplasmosis will be used to illustrate important aspects in the development of resistance. It will also discuss important points to protect the future usage of this important drug group in animal production by sound treatment advice and flanking measures.

Resistance mechanisms in macrolides

First of all, it is important to elucidate the usual working mechanisms of macrolide antibiotics versus mycoplasma. Bacteria in general have different defence strategies against different antibiotic groups.

One example is restricting access by modifying the entryways into the bacterium itself.

Another mechanism is the usage of efflux pumps that facilitate the expulsion of antibiotic molecules form the bacterial cell.

Also, antibiotic molecules inside a cell might be either enzymatically inactivated or their target might be modified in such a way that the antibiotic no longer recognises its target location. This is known as target site modification.

In mycoplasma, resistance is usually established by the usage of efflux pumps, by target site modification or by direct drug inactivation.

Furthermore, mycoplasmas are intrinsically not sensitive to antibiotics targeting the cell wall, as they do not possess a cell wall. Hence, the usage of β-lactam or glycopeptide antibiotics to counteract mycoplasmosis is pointless.

To be more specific, macrolides bind within the tunnel of the 50s ribosomal subunit where cell proteins are being produced and there interact with several messengers and subunits. Several point mutations in said subunits have been uncovered in macrolide-resistant Mycoplasma gallisepticum (Mg) isolates from Egypt, China and Israel. Mutations in the same mechanism but in different locations on the genetic code have also been reported for Mycoplasma synoviae (Ms) leading to the substitution of several amino acids in the nascent protein.

There is a correlation between a reduction in the susceptibility to macrolides and lincosamides with the substitution of amino acids in the 23s rRNA alleles in Ms, making it also intrinsically resistance to 14-membered macrolides such as erythromycin.

Strains of Ms with reduced susceptibility to macrolides and lincomycin were reported in studies performed in many countries between 2004 and 2014.

How to test for resistance

After having briefly discussed how resistance develops it is important to understand how the resistance status can be evaluated, especially in difficult to isolate mycoplasma. The effectiveness on antimicrobials can be assessed by in-vitro susceptibility testing, used to determine the MIC (minimal inhibitory concentration) and MBC (minimum bactericidal concentration) of an antimicrobial agent.

Due to their slow growth, very small size of colonies, and complex growth requirements, standard procedures, such as the disk diffusion method used to test the in-vitro susceptibility of other bacteria, are not recommended for mycoplasmas of poultry.

To test for resistance of mycoplasma the minimum inhibitory concentration test (MICs) may be performed in solid media or in broth. To perform this technique, it is necessary to take many things into careful consideration, such as the selection of culture media, the concentration of the inoculum, dilution of the antimicrobials and the incubation period.

To describe the method briefly, a final dilution of the mycoplasma strain between 10^3-10^5 CCU (changing

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colour units) shall be inoculated in a 96 well plate where the antibiotic dilution already has been applied. The mixture is incubated at 36 ± 1°C for 24, 48 hours or up to seven days depending on the strain. Several studies on MIC values for mycoplasma strains in poultry exist.

Fig. 1. demonstrates the ranges of MIC values of different strains analysed together versus the most used antibiotics to treat mycoplasma infection. The isolates were collected from numerous countries worldwide: Argentina, Egypt, Italy, Japan, Spain, The Netherlands and the USA.

When and how to use macrolides

As mentioned before, macrolides are one of the most widely chosen options to treat a mycoplasma infection in poultry. However, the macrolide family has many members and so it is necessary to choose the correct drug according to the situation at hand.

Based on MIC and field results, one of the newest macrolides on the market, Tylvalosin, is a targeted antibiotic with one of the best efficacy profiles versus mycoplasma.

In order to act within the bounds of antibiotic stewardship sound treatment advice and measures flanking therapeutic interventions need to be followed.

First of all, in diseases with few distinct pathognomonic clinical signs such as Mg or Ms, lab support is needed to confirm the clinical suspects. A serological test such as ELISA is a very useful tool to check if the flock is positive, negative or to acquire useful information on their vaccination status. PCR can be used as a confirmation tool in case of a positive ELISA or as a direct diagnosis technique in case of clinical signs with a negative serology.

Several typical treatment scenarios exist. One example is the treatment of vertical transmission as it often occurs with poultry mycoplasma. Vertical transmission from parent to offspring is responsible for continuing the mycoplasma cycle in poultry production.

Therefore, controlling mycoplasma in breeders is a prerequisite for interrupting vertical transmission. In mycoplasma positive breeders flocks, this may be achieved using regular treatments every 21 days. If breeder treatment cannot be ensured on a reliable basis, it is advisable to medicate the day-old chicks in the first days of life in order to reduce the mycoplasma transmitted vertically from positive parent flocks. In other types of poultry production, like layer production, the treatments can be made when production problems or clinical signs are observed. To ensure biggest success in this given scenario a sensitive diagnostic tool should be employed, allowing for immediate pick up and commencement of treatment at the first observed positivity.

PCR lends itself perfectly to this approach. Any serological test has the inborn disadvantage of showing the body's reaction to a present antigen rather than detecting the antigen directly itself. If PCR or qPCR as a monitoring tool is cost prohibitive, the rapid plate agglutination has the potential to detect earlier antibodies developed than for example regular ELISAs.

Another scenario under the antibiotic stewardship approach is the combined use of mycoplasma live vaccines and antibiotics in the same flock.

In an already positive flock, a tylvalosin treatment given at a defined time point before a live vaccine will ensure that the vaccine strain may develop its optimal protective effect during the flocks life. In this way, the live vaccine will establish itself in the flock without any interference by an already present field strain. In another scenario, antibiotic treatment can also be given after a live vaccine. For example, when it is proven by laboratory diagnosis and the onset of clinical signs that a field strain has taken hold in the flock.

Conclusion

The mission is to safeguard antibiotics for the future use in animal production. It is of paramount importance for the continued usage of antibiotics on a global scale to use them optimally. Resistance development must be counteracted by the correct use of modern drugs in a responsible way.

Even with the many advances made in management and vaccines, animals will continue to succumb to bacterial infections and get sick. When they do, antibiotics must be available for treatment also on welfare grounds. It is of utmost importance that the limited available antibiotics maintain efficacy for as long as possible into the future.

The best way to do so is to use antibiotics responsibly in both humans and animals. This includes using antibiotics in defined scenarios with careful attention to monitoring and generally sound management practices in poultry production. ■