Title: Introduction to Antimicrobials and Antimicrobial Resistance

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- [Dr. Tim] Hello and welcome to this introduction to antimicrobials and antimicrobial resistance. And this is part of the Farm Vet Champions. Modular course on antimicrobial stewardship. And I would like to point out that I am primarily a small animal vet. Although I have a lot of interest in antimicrobial resistance and antimicrobial stewardship and I would like to acknowledge the help from my colleagues, Rob Kelly and Amy Jennings at the university of Edinburgh and Fiona Lovatt, And Pam Mosedale's help in carefully and helpfully reviewing this presentation and providing me with some farm specific examples. So these are the learning objectives. And what I'm hoping to do in this brief introduction really is explain what's happening. And the threat that we see from antimicrobial resistance, how this is arisen how this is spreading, why this is a one health problem and then help you begin to understand the drivers for your own antimicrobial use. Which will be explored in other modules in this course. And then introduce the concept of the microbiome and introduce the concept of the majority of the time what we're treating is not in inverted commas an infection something that's been acquired and can be got rid of. But what we're seeing is a dysbiosis of the normal commensal population of bacteria and how really we need to be refocusing efforts away from antibiotic therapy and into maintaining a normal, healthy, diverse microbiome and preventing the dysbiosis. And in this way, we can maintain animal health and high levels of production and high levels of animal welfare without using antimicrobials and therefore come back and begin to reverse this relentless drive that we've seen towards antimicrobial resistance.

So you'll see the term antimicrobial and antibiotic bandied about a bit interchangeably and I have to hold my hands up here and say I'm as guilty as the next person for doing this. But essentially an antimicrobial is anything that's going to kill a micro-organism. And in terms of treatment, we thinking about the anti pathogen activity towards bacteria fungi viruses and protozoa. But in technical terms really shorthand really, we'll be talking about the antibacterial therapy. And then antibiotics, these are substances that are produced or derived from substances produced by bacteria that have an inhibitory impact on other bacteria. And antibacterials, this is where the terminology gets a bit confusing. Basically do the same thing but these are purely synthetic compounds. And then when we talk about disinfectants or biocide what we're looking here really is surface active agents. So agents that we could use on inanimate surfaces for cleaning, but also on the skin and elsewhere for this infection. And one of the important things to understand is that many of the antibiotics are derived from natural sources simply because antibiotics and antimicrobial resistance have been around for hundreds of millions of years because bacteria use these to compete with each other, for environmental niches. And there's just been this low level, arms, race going on between developing antibiotics and developing resistance. And what we've done is come in and upset the Apple cart by starting to use these compounds in industrial quantities swamping out the susceptible bacteria and giving the resistant bacteria a huge competitive advantage. The advent of antibiotics had a massive impact on both human health care and animal health care. And just after the second world war was

known as the golden age of antibiotics where you would see these dramatic cures with just one pill, one day's worth of treatment which we just don't see anymore. And this is enabled a lot of what we would now regard as modern health care. And one of the threats that we see is the increase in antimicrobial resistance unless checked could render a much of what we regard as modern human and veterinary health care on feasible or impossible because of the risk of resistance.

Now I do a lot of lecturing with a guy called Billy Malcolm who is a pharmacist in Scotland and one of the leads for antimicrobial resistance and stewardship strategies for the for the Scottish NHS. And he relates these stories that when he was a small boy in the late 1950s, he contracted pneumonia, a little over a decade earlier that would have been a death sentence almost. And he responded very quickly to antibiotic therapy. One of his daughters was born by caesarean section against a procedure that carries a very high risk of infection and mortality without the use of antibiotics. His goddaughter is a premature baby, spent a lot of time in a premature baby unit, contracted an infection. It was able to be treated with antibiotics. Recently, his father has had heart valve replacement operation the sort of thing, again, this only possible now with antibiotics and the list goes on. In my own family, my mother was born just before the second world war. And as a toddler almost died of what would now be regarded as a routine antibiotic treatable infection. And a few years ago my father contracted an MRSA urinary tract infection. So within the lifetime of my parents we've gone from a pre antibiotic era to staring into the abyss of a post antibiotic era. And that's not great. So how does this occur?

So as I said earlier, this is in effect a natural phenomenon. So bacteria have been producing antibiotics and have been co-evolving antimicrobial resistance now for hundreds of millions of years to give themselves advantages in niche competition. And this can be the resistance can be divided into what we call inherent or acquired. Now inherent resistance is simply where the antibiotic is never going to work because either can't penetrate the the bacterial cell wall. So a good example here would be the standard benzol penicillin. That huge benzol ring means that it can't penetrate the cell wall of gramme negative bacteria which is why this drug is a narrow spectrum agent and works on gram-positives only. And I've put some other examples down there for you. And then you can have acquired resistance. Now, bacteria are very, very good at mutating. They have a very high mutation rate as prokaryotic organisms compare to eukaryotic organisms, which has everything else in the tree of life, really. And so these mutations arise purely by chance, but then if they give that bacteria selective advantage, they can spread within the population. And this is what the antibiotics do. And what are the problems with procaryotes again is that they're very good at exchanging DNA. So very good exchanging these mobile packets of DNA which can encode for antimicrobial resistance. And these are known as plasmids. Particularly, in gramme negatives, they seem to be much more promiscuous compared to gram-positives in this way they can exchange these plasmids and a single plasmid can contain multiple antimicrobial resistance genes conferring resistance, to a range of related and unrelated classes of drug in one go. And this exchange is particularly important where you get lots of bacteria, these microbiome soups that are mixing together. That's a really good example here would be in the gut or the rumen of farm animals where you get these massively complicated bacterial populations.

Now, this is work done by Vanessa Smith, when she was doing her PhD with me at the university of Liverpool. And what she did was a beautiful piece of work where she looked at dogs that were being given antibiotics didn't matter what for, of different classes. And then she took various samples from them to look for population structures and antimicrobial resistance carriage before treatment. She then tested this again after treatment and no matter what she looked at, the prevalence of those resistance parameters went up and it took about three months for this to get back to baseline again

three months off antibiotic to get to boat back to baseline. And in some cases it never did those animals became permanent carriers of antimicrobials resistance. This is just a brief diagram to show that the various different targets for different classes of antibiotics. And that don't worry too much about the technical details here, but it just shows you the breadth of ways that resistance can emerge. And this can be by altering the cell targets. So an example would be MRSA and MRSP which have an altered penicillin binding protein in cell wall construction, which means that the penicillin and cephalosporins won't work because they don't recognise or bind to that altered penicillin binding protein. We can have changes in target proteins again so that the antibiotics don't bind to these. We can have efflux pumps. That mean that when the antibiotic enters the cell it's just pumped out again and so on. So there is no use in thinking about if we just develop an extra technical special antibiotic it is somehow going to be evolution proof bacteria survived on earth for something like 3 billion years, they've survived everything the earth and the universe can throw at them. Whatever we think of in terms of treatment evolution is smarter than we are. And the way to slow down the development of resistance is by using antimicrobials less often and using them smarter.

So, again, it's just a diagram here to show you the various resistance mechanisms there. And again, examples of these have been detected to a very wide range of antibiotics, and there are now multiple resistance genes even targeting single antibiotics or antibiotic classes, meaning that the way of evading these by developing new drugs is becoming harder. Now see the problem of the transferable resistance that I mentioned. So this is a particularly common amongst gramme negative bacteria. It is less common amongst gramme positive bacteria because they actually don't like taking up foreign DNA and they actually will often destroy it. So it happens very rarely amongst gram-positive bacteria. And then what you'd get is clonal expansion of those resistance clones, and transmission between individuals. But the big worry about gramme negatives is you are getting this transmission within these mixed populations. And one of the problems that we see, is not so much that it's this infection that becomes resistant or that that can happen with fast evolving things like pseudomonas, but it's the next one. So what you've done, with systemic treatment for example is select for carriage for resistance amongst the coli or enteroccocci or Klebsiella within the gut, within this VAT we can get exchange of those materials and lo and behold the next infection that you see is going to be the antimicrobial resistant one, that was selected for by the earlier course of treatment. And this is becoming a huge problem across all species, so for example, in hospitals, human medical hospitals, and in companion animal veterinary hospitals, we're actually seeing a reduction in resistance amongst gram-positive organisms. So mostly MRSA and MRSP, but what we're seeing is an increase in resistance amongst the gramme negative organisms, particularly extended spectrum beta-lactamase producing an AmpC producing coliforms that are highly resistant to penicillin cephalosporins and sometimes other classes of drug as well. And one of our challenges for the future is what are we doing that is working for the ground positives but not the gramme negatives, and how can we improve that.

So, I mentioned this a little bit earlier on as well. These plasmids acquire little packets of DNA through conjugation. And so, a single plasmid can start to collect lots of little packets of DNA in coding for resistance. If these are conferring a competitive advantage in terms of resistance to that organism. And then the problem that you can have is that that plasmid is passed on to the next bacteria as one package. And therefore in one easy move, you suddenly go from a very susceptible bacteria now to very highly resistant bacteria. And the single plasmid can contain resistance genes not just a one class of antibiotics, but to multiple classes of antibiotics. And the problem then is, because you're selecting for the survival of that resistant bacteria by using any one antibiotic, you are coast selecting for a much broader range of resistance. So it is not sometimes as simple as

saying, well, if we only use this drug will only sell it for resistance to this drug, it just doesn't work like that. Now the good news, is that if we remove that selective advantage the resistant bacteria can be out competed. And this is because bacteria don't really like to have lots of DNA. Most of them will compete for niche advantage by being very streamlined, very fast growing and very fast dividing. And that can be inhibited by having a lot of DNA around that you don't need. So, in the absence of that selective advantage conferred by the antibiotics you will find that the susceptible bacteria can outgrow and out-compete their resistant cousins. And this is why the absence of antibiotics, we can see a gradual loss of resistance. And this is why one of the key aspects of antimicrobial stewardship is to use fewer antibiotics and use them more cleverly. So in other words, less antibiotic and smarter use.

We do see some problems with persistent carriage. I mentioned this and finishes Smith's PhD earlier on. And again, this is a particular problem where we have chromosomal cassettes or plasmids that are carrying relatively few antimicrobial resistance genes. If the genetic cost of carrying that is not huge they can become established as part of the normal commensal micro flora. And this is a particular problem with some of the methicillin-resistant staphylococcus isolate some of the MRSA and MRSP isolates. Now, this is the big challenge that we face is that, the vast majority of antibiotics were developed in a kind of mid to latest 20th century. So in other words, before the 1970s with very few classes of drug developed since then and in fact, since the 1970s, there have been no you've got relicensed products developed and it is likely that if genuinely new classes of antibiotic are discovered in the near future they are probably going to be restricted for human use only. And it is unlikely that they will be licenced for animal use particularly for food producing animals. So, for the foreseeable future, we have what we have, and if we want to preserve the efficacy of these drugs for the future, we have to exercise greater stewardship.

This is an infographic from the Laura Neil's report that was commissioned some years ago. Estimating that the worldwide impact of antimicrobial resistance with something like 700,000 people a year dying shot the world of on treatable infections. And if we do nothing with current trends by 2050 that's estimated to be 10 million. And this is a true one health problem as I mentioned earlier, because you know between humans and animals in the environment, we have very similar bacteria, if not identical bacteria we use very similar drugs at least of the same class and sometimes exactly the same drug and the genes that confer the resistance are exactly the same. So there is nothing to be gained by pointing the finger and blaming farmers or vet or doctors or human patients. We've all behaved badly in the past. We all need to do better. We all need to work together. This is one resistance ecosystem. This is kind of quite old data now, but it's just to show that these resistance genes move very readily across species. So this is looking at staphylococcus Oreos colonisation among vets, and these were done at conferences. So, usually more than 24 hours away from clinical practise. So this was colonisation rather than transient courage and a substantial number. So something in the order of 10% to 15% of the vets that were sampled were MRSA colonised. So that is way more than you would expect to see in the normal population so it's an occupational risk for us and interestingly the strain correlated with the type of practise. And you can see this amongst horse owners, you can see this amongst farmers as well.

Now, the good news is that farm animal practise has been ahead of the game, when it comes to reducing antimicrobial use. And since 2015, there has been a very substantial and sustained decrease in overall antimicrobial use. And especially in a decrease in the use of water called highest priority, critically important time to microbial, so HPCIS, which are the ones deemed most critical for human health. So the group we really want to be protecting. And some of the strategies that have led to this change will be looked at in other modules within this course. Now, the drivers for

antimicrobial use. And this is one of the key areas to look at in terms of reducing the use is all really about the interaction between the farmer or the farm worker and the veterinarian. And this is governed by things like concern for animal welfare, concern for the profitability and the impact on the economics by using antibiotics or by not using antibiotics, communication and trust but also a big driver is practise culture. So, nobody likes to be the outlier and people coming into a practise will tend to adopt the practise behaviours there. And this is why critical analysis and clinical audit of practise use can be... And then adopting a team approach can be really critical in evaluating therapeutic strategies and reducing use. And again, this is the sort of thing that will be covered through this course. And this is one of my favourite sayings, so again if the infection comes back, that animal does not have a cephalexin deficiency or an amoxiclav deficiency or whatever, your poison of choice is.

The vast majority of infections we treat do not involve primary pathogens. So mycobacteria would be a good example of a primary pathogen. It's not part of the commensal micro flora its job is to create disease, which it can do very well. In reality, many of the infections we treat, if you think about it involve a dysbiosis of what is otherwise a normal diverse population. We see a skewing towards potentially more pathogenic organisms, and you see the diseases as a result of that. And what we have to do is look for the underlying reasons of that, which can be underlying health conditions of particular importance to production animals would be the environment. And then in the future, really what we want to be doing is looking at strategies that maintain and promote that healthy microbiome diversity to prevent that dysbiosis and the health problems in the first place. As I said earlier, we're gonna, you know I hope as part of this introduction to get the concept of microbiome diversity or introduce you to that concept of microbiome diversity and depending on the microbiome that you're looking at depending on how it's measured and defined and so on for every human or animal cell in the body there are between three and 10 microbial cells. So in fact, our microbiome in numbers of cells massively outnumbers the endogenous cells here I said this earlier. So, a lot of the time we've got to get away from this idea of infection because it encourages antimicrobial use because you go down the rabbit hole then of thinking infection it's been acquired, it can be treated and got rid of and reality for many cases that's not the case because we've got commensal bacteria either somewhere where they shouldn't be or we've got this local dysbiosis. And one of the problems with recurrent antibiotic use is if you systemic drugs you are not just treating the site of infection, you are treating the whole of the microbiome and that can have downstream consequences in terms of antimicrobial resistance, remember I said earlier you select the resistance, which can then come around to bite you in the future. But at the same time, you will be reducing microbiome diversity which can have longterm impacts on production and health. And with the help of Rob, Amy and Fiona I've tried to put together a few examples here.

Again, this is not really my field, so apologies if I've completely misunderstood things here. But my understanding is that the problem of watery mouth and diarrhoea in lambs is associated with the E. coli. E. coli is a normal of the gut as part of this fantastically complex and diverse gut microbiome. If however, the gut microbiome becomes less diverse and skewed towards E. coli particularly the potentially more pathogenic E. coli you will then see the clinical signs associated with this. And the risk factors are all to do with management because of what they're doing is allowing the dysbiosis to take place. And antibiotics can work, but what you're not doing is eliminating somehow pathogenically acquired E. coli what you're actually doing its sounds counter-intuitive is knocking the head off if you will, of this high on stable and a dominant E. coli population and allowing the restoration of the normal microbiome. But the consequences, every time we do that is, we will see less diversity longterm which kind of impacts on health and production. We're also selecting for resistance which means we can't keep doing this forever because there will come a point where it

will stop working. And this is work by Jenny Duncan from the university of Liverpool. It was published in the factory record quite recently. And what they did is looked at the resistance parameters in nine different farms. This is just one of the diagrams on the paper and you can see on some of the farms here, actually, most of the E. coli life isolates they're seeing are still susceptible to relatively first-line and routinely available antibiotics. But in two of the farms here we were beginning to see a very high level of resistance to the most commonly used first-line unlicensed antibiotics. And, if these farms don't look out within a very short period of time, they're going to run into a situation where these infections become untreatable.

Another example would be strep uberis mastitis. So this is something that's relatively easy to identify. You can use monitoring techniques to pick it up, cytology of milk stripping show the classic chains of bacteria. And then this can also be identified by culture as well. And again, this is an environmental organism so it's not generally part of the normal microbiome, but it's an opportunist. Again, it's not a primary pathogen. So if it's becoming a recurrent problem there are reasons why, and these need to be addressed long-term to get this population under control rather than trying to rely on blanket antibiotic, topical antibiotic therapy particularly dry cow therapy, because there will come a point where the dynamic relationship between the cows, the milking machines and the rest of the environment will lead to resistant population on that farm, and it will become harder and harder to treat. So, again, this is areas that I won't go into a lot of detail now, because this is going to get covered in the other modules, in this course but it's about using antimicrobials less often and smarter so that our treatment is targeted. We're using clinical science, cytology and culture to precisely identify the infection. So we're not using blanket treatments, we're not using just in case or speculative treatments. And then we're more accurately targeting therapy towards the individual and kind of seems basic but get the dosing right. And then looking at management. So working with farmers, looking at the overall husbandry and management with the eventual aim of reduce and replace the antimicrobial therapy. To reduce this march towards resistance. This is, again something that will be covered later on, and this is a big, big push in human therapeutics particularly for viral infections. If it's not a bacteria antibiotics, won't work we need to precisely identify what we're dealing with and treat appropriately. So this involves the importance of the diagnosis again.

It's always then worth taking a step back because even in humans, bacterial infections, verge from the trivial that can be easily managed by symptomatic therapy to the very serious that need intensive care unit and intravenous antibiotic therapy and then everything in the middle. And it is always worth taking that step back and just having a moment to think how do I need to treat this? Is it severe enough to warrant systemic antimicrobial therapy or even antimicrobial therapy at all? Are there other ways to manage the problem? And then we talked about the importance of managing the dysbiosis earlier. And this is by looking at general health, husbandry bias securities all of the factors that are leading to that dysbiosis in the first place, and trying to avoid them trying to reduce our antimicrobial use and reserve that for the animals that actually need it. And then in the future, and I think, unfortunately at the moment, although we've got good evidence for the importance of a healthy microbiome, the evidence for the use of these prebiotic, probiotic at microbiome transplant techniques is not quite there. Now, there's good evidence for some of these approaches in humans. There's beginning to get evidence in companion animals in farm animals. And I think this area is going to become very big in the next few years but I think we also have to await good evidence and seek good evidence for using these approaches, so we need to know that they're going to work. So, I hope that was a useful oversight into the threat from antimicrobial resistance how this arises, the impact of antibiotic therapy. And then beginning to think about the microbiome and dysbiosis, rather than this simplistic view of infection and steps that we can start to take to prevent that dysbiosis happening so that we can preserve health and production without using antibiotics.

Because if we don't do this, we will lose the efficacy of these drugs and that will affect us all. It's going to affect me, you, our loved ones the animals that we care for. Thank you.