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Resistance build-up a worry

Over-reliance on antibiotics means their effectiveness is increasingly under threat

As a young vet student I saw practice with the legendary Bob Crowhurst in Newmarket. During a discussion on the appropriate use of antibiotics in the horse, he recounted the fact that for several years during the war he was in charge of an army remount station, I think in the Sudan, where his entire repertoire of drugs included salt, water, and any combination of the above!

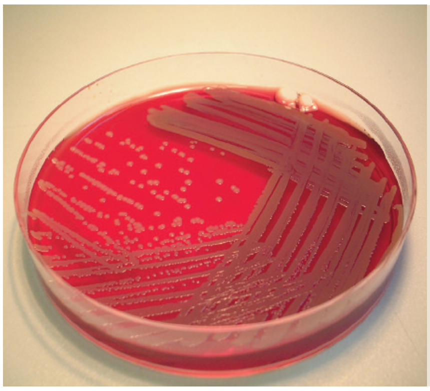
To someone who had grown up with antibiotics being a normal part of clinical practice, it was astounding that any injured horse could be saved without recourse to their use. We have to remember, however, that penicillin, even for use in man, came in only towards the end of the war and had certainly not reached equine practice at that time.

We now live in a world where we expect severe infections in the horse to be curable, and where minor infections such as infected wounds and cellulitis in the limb are so routinely cured that they are regarded merely as a nuisance, and of no great significance. This state of mind only exists, however, because of the extremely effective use of antibiotics we have come to expect, and this effectiveness is increasingly under threat because of drug resistance in the bacteria which we are trying to attack and kill. If we are not careful, we risk a return to the days when all we will have to treat infected wounds is salt water.

Why do bacteria matter?

Bacteria exist by the millions and billions almost everywhere on the planet. They cover our skin, live up our noses, and even form a large proportion of our faeces. Similarly, in the horse, many bacteria live within the digestive tract and perform a useful function in aiding the horse's breakdown of what is essentially indigestible cellulose, the main component of plants. No animal species possesses the enzymes to digest cellulose (even termites who live on a staple diet of wood have small 'farms' of bacteria and simple cell organisms to do the job for them).

As well as these bacteria, normally called the gut flora, the horse also has bacteria over most of its skin surface, including many species of staphylococci, which for the most part are present in small numbers, and live in harmony with the animal. Other bacteria are commonly found in the nasal chambers and throat, where they live quite harmlessly until the animal is diseased. These bacteria pose no threat normally, but, introduced into wounds, or by gaining access to the bloodstream, they can set up potentially life threatening infections.



A typical bacterial culture in a Petri dish. Here a pure growth of one organism, a Staphylococcus sp., is apparent. Even though bacteria are so tiny they are invisible, when they multiply rapidly in an incubator, they form colonies which stack up until we can see them with the naked eye. Each of the yellow 'lumps' is just such a colony, composed entirely of bacteria

Why is drug resistance present in bacteria at all?

Bacteria have been around for millennia but, as we know, antibiotics have been in existence for less than a century, so why and how have bacteria managed to become resistant to them?

A bacterial antibiotic sensitivity test: a single bacterial colony has been picked off the culture plate, mixed with a small drop of fluid, and then wiped over the whole surface of a new plate to produce a 'lawn'. Each paper disc contains a different antibiotic, which diffuses into the agar gel and prevents growth of any sensitive bacteria in a zone of the 'lawn' around it. Here, we can see the bacterium concerned is totally resistant to two different antibiotics

To answer this we have to look at the source of the antibiotics. Every school-level scientist knows the story of Alexander Fleming's discovery of penicillin, when he found that bacterial colonies were inhibited from growing on agar gel plates, if these had been contaminated with moulds and fungi. He postulated that the moulds must be secreting a chemical into the gel which inhibited the bacteria from multiplying, and he was right.

Penicillin derives its name from the fact that it was obtained from the fungal organism Penicillium. All the antibiotics we use are in fact derived or styled upon naturally occurring molecules that exist in nature to inhibit bacterial growth. Fungi want to have the food source to themselves, not to share it with bacterial rot, so they kill off the bugs.

Although penicillin has only recently been put into a bottle, and used in medicine, bacteria have been exposed to its action over almost the same length of time as their own existence. This has allowed occasional genetic mutations, which confer resistance to attacks by penicillin, to thrive in certain situations and these organisms become part of the normal bacterial colonisation of the animal.

If antibiotics are not used, then they are not in any way advantaged, and as long as they are not disadvantaged they will simply remain a small part of the population. However, if antibiotics such as penicillin *are* used, then this immediately gives these resistant organisms an advantage. The rest of the bacteria that have no drug resistance will be eliminated, leaving only the resistant strains within the horse, which will then be able to multiply rapidly and fill the sites now vacated. The horse in this way becomes a potent source of drug-resistant species of bacteria. This happens to some extent every single time we use antibiotics. The more they are used, the more we are selecting for resistance.

How is antibiotic resistance detected?

The one good thing about bacteria is that they multiply rapidly given a warm environment and a supply of nutrients. This is put to our advantage in testing antibiotic sensitivity from organisms isolated from clinical infections in practice. A small sample from the infected site is spread out on a shallow dish filled with agar gel, often incorporating sheep blood cells as a nutrient source for the bacteria (*see image top left*). After initial 'plating out' to detect the types of bacteria present, each individual bacterial type can then be made into a thin 'soup' and flooded over the surface of another similar agar plate. This will grow a 'lawn' of bacteria, which are detectable to the naked eye once they stack up on top of each other, as a white film. At the time the lawn is seeded, a set of small paper discs impregnated with different antibiotics is placed on top of the agar. The antibiotics diffuse into the gel at the same time as the bacteria begin to multiply and, obviously, if the bacteria concerned is killed or inhibited by the antibiotic, then a zone of no growth will appear around that antibiotic disc (*see image below left*). In a nice warm incubator, bacterial growth is so rapid that it usually takes only a day for us to get the answer as to whether our antibiotic will be effective or not, and to flag up potential drug resistance.

Is antibiotic resistance increasing in the horse?

To answer this question, a team from the Royal Veterinary College in London, headed by Dr Imogen Johns, looked at two different periods of six years, spanning 1999-2004 and 2007-2012. They looked at all of the bacterial isolates obtained from both the horses dealt with by local vets and their own hospital patients during this time, and compared resistance levels between the two periods.

They focussed on two main bacterial types: *Streptococci*, which we know from sore throats, and *E.coli*, which we know from sporadic food-poisoning outbreaks. The former is a common target species for antibiotic use in the horse, commonly implicated in, for instance, infected cuts and respiratory diseases characterised by coughing. *E.coli* in contrast is much less often a target species, but is there in the gut in huge numbers, and so gets bombarded with antibiotics whenever they are used. It thus acts as a good 'sentinel' for growing resistance development in general. The results were worrying.

There were significant increases in multiple-drug resistance in the *E.coli*, which would have seldom been the target of treatment, ramming home the message that when one uses an antibiotic, it affects all the bacteria in the horse, not just those in the septic focus. In the *streptococci*, resistance had also increased, sometimes alarmingly. One antibiotic in particular, enrofloxacin, which racehorse trainers will know as the brand 'Baytril', was very concerning. In the first six-year period, resistance was 0% of isolates. In the second trial period it was 63%. Worse still, this antibiotic is one of a group known as fluoroquinolones, which is particularly vital for use in man, and one which vets have continuously been asked to resist using, because of the fear of developing resistance.

Looks like we're too late on that one, but even worse is the fact that 'Baytril' is not even really intended for the horse. The oral form is marketed to put in the drinking water of chickens, a much more potent source of supply of resistant bugs to man!

To put things into perspective with regard to the position in the horse, antibiotics here tend to be used only on a single-animal basis. This is in stark contrast to the position in farming, where antibiotics are often used in blanket medication of whole flocks or herds.

This is largely to treat or prevent diseases that would otherwise occur as an inevitable result of the high-stocking densities used to maximise the profit in livestock production. For instance, few people realise that the chickens we all eat from the supermarket live continuously on one type of antibiotic just to enable them to be raised inside a broiler shed.

Without the constant medication with drugs to stop coccidia killing the chickens, they would quickly die, living as they do for their whole life on bedding composed mainly of their own droppings. In the first study of its kind, researchers recently estimated total usage of antibiotics in farming in 2010 at 63,000 tons – and this is almost certainly a massive underestimate of usage now. This is around twice as much as all the antibiotics produced to fight human disease worldwide.

We know that some bacteria are quite happy to jump species, and it's this constant selection pressure via blanket medication in farming that probably poses more of a risk to us than our own use in an individual sick horse. Nevertheless we can't afford to be complacent, and everyone in our industry should do everything they can to preserve this vital line of treatment.

What can we do about it?

Dr Mark Holmes, a reader in Microbial Genomics at the University of Cambridge School of Veterinary medicine, has been investigating multiple drug resistance for over ten years. His group are particularly interested in resistance among *Staphylococci*, a group of bugs which include the killer MRSA organisms we all live in fear of catching as hospital 'super infections' following surgery.

He comments: "On the whole we're very lucky in veterinary medicine that we don't see a lot of clinical problems with antibiotic resistance – occasionally we get a condition in a particular patient that is not responding as we might expect it to, and we switch antibiotic, and most times we then get a response. But you know, so far, we've just been lucky.

"The most important thing, particularly within the veterinary profession, is that we as vets retain the power to prescribe across a broad antibiotic range. What we've got to do is to demonstrate, particularly to the medical profession, that we're behaving responsibly. Otherwise, quite reasonably, they will say, 'If vets are not going to behave and prescribe responsibly, why should we risk giving some of these antibiotics that are of critical value in human medicine to them at all?'"

He adds: "I think that's why we've got to be pre-emptive, and got to get our house in order from an early stage. The thing that we can do is to avoid using broad-spectrum antibiotics when we don't need to use them.

"It's important that we don't resort to using antibiotics automatically, especially when they're not indicated, and it matters that we look at the likely antibiotic susceptibility of the bacteria we are targeting, and then prescribe appropriately."

We have become so used to infection being curable in our horses, it seems inconceivable this situation could ever end, but the trends described here are a worry, and a worry all of us need to take seriously. The thought of returning to the days when bottled antibiotics did not exist, and all we will have to treat infections is salt and water, is not an appealing one.