# PHARMACOKINETICS - ANTIMICROBIAL SENSITIVITY AND RESISTANCE

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**Summary**

*In the UK, approximately 40-45% of the therapeutic use of the 459 tonnes of antimicrobials used are administered to pigs. Much concern has recently been expressed over the development of resistant bacteria in pigs and the possibilities of the spread of antimicrobial resistance to man either by zoonotic pathogens or by bacterial contamination of food. To help the veterinarian and producer choose the antimicrobial more effectively, so that therapeutic response is improved and resistance reduced, the use of pharmacokinetics and pharmacodynamics, in relation to the minimal inhibitory concentrations against various pathogenic bacteria, is discussed and data is presented.*

## Introduction

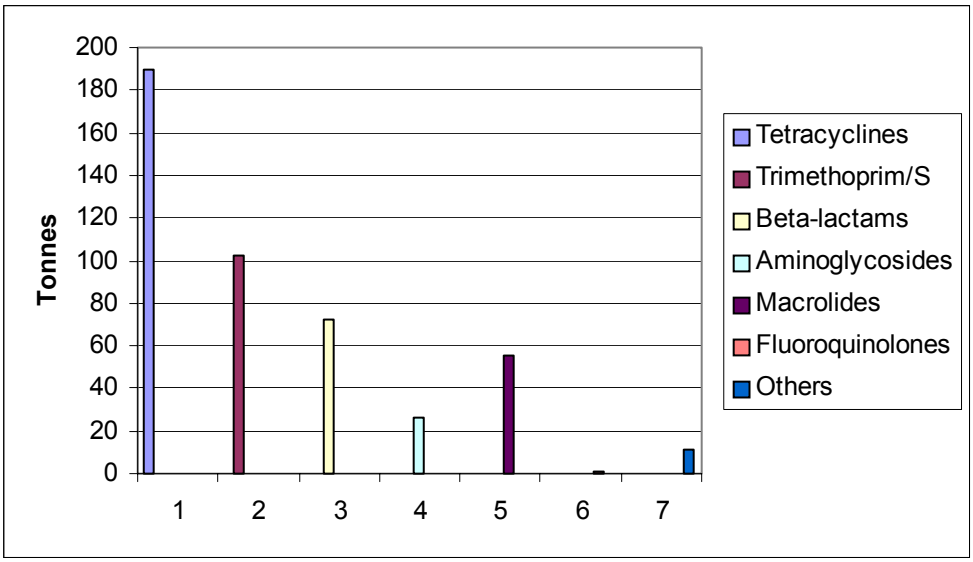
In the fight to keep pigs growing healthily and well, in the face of severe respiratory and enteric disease challenges, it has become common for veterinarians to use antimicrobials to treat acute infectious disease or, more commonly, prevent them from developing by strategic use. Farmers want to see their pigs do well and are prepared to use cost/effective medications to achieve this. Consumers also want cheap, good quality meat without antimicrobial residues, or zoonotic pathogens, such as salmonella and campylobacter, and also free from resistant bacteria, which may compromise their own health.

Unfortunately, the use of antimicrobials tends to cause bacterial resistance and thus there is a balance between the need to maintain an animal's health, welfare and productivity with the consumer's requirement for uncontaminated meat. This is monitored by regulators in-between, who try to control the system and maintain the equilibrium. It behoves veterinarians and producers alike to use the products responsibly and prudently, although cost should restrict any flagrant overuse. By improving understanding of the products used, their pharmacokinetics (PK), pharmacodynamics (PD) and relating them to the antimicrobial susceptibility of the pathogenic bacteria, their effective use will be enhanced, their therapeutic benefits maximised and, at the same time, resistance development minimised.

## Antimicrobial usage in the United Kingdom

The Veterinary Medicines Directorate (VMD) has been assembling the data on the quantity of antimicrobials used in the UK over recent years. In 2001, the sales of therapeutic antimicrobials were 459 tonnes of active substance (see Graph 1), with 39 tonnes used in companion animal medicine and 420 tonnes in farm animal medicine. An additional 43 tonnes are still used as growth promoters.

**Graph 1 - Therapeutic use of antimicrobials veterinary medicine in 2001 by product group**

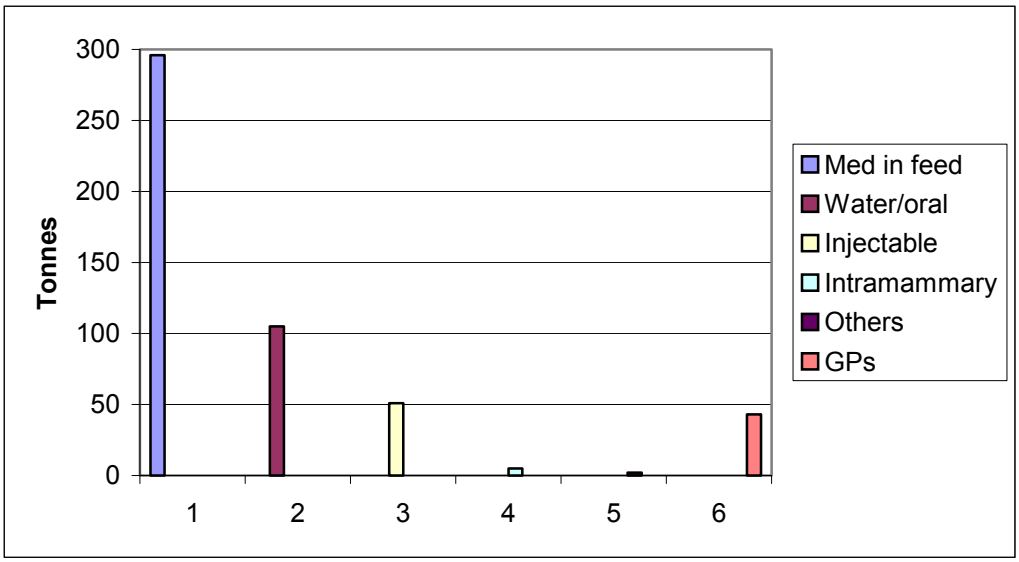



Source: VMD, 2003

Tetracyclines remain the major antibiotic group, with in-feed chlortetracycline predominating. This is followed by trimethoprim/sulpha combinations and beta-lactams (penicillins and synthetic penicillins such as amoxycillin) and macrolides, which are predominately tylosin in-feed for pigs. Aminoglycosides, such as neomycin and apramycin, follow with a combined group of 'other' compounds including lincomycin and tiamulin. Fluoroquinolones, which are very important products in human medicine, are not used widely in veterinary medicine, especially pigs, at about one tonne.

If usage is further analysed by route of administration, medication via the feed is the largest route (64%) followed by soluble and other oral preparations (23%). Injectables account for 11% and intramammaries only 1% (see Graph 2).

**Graph 2 - Antimicrobial use via route of administration in 2001**



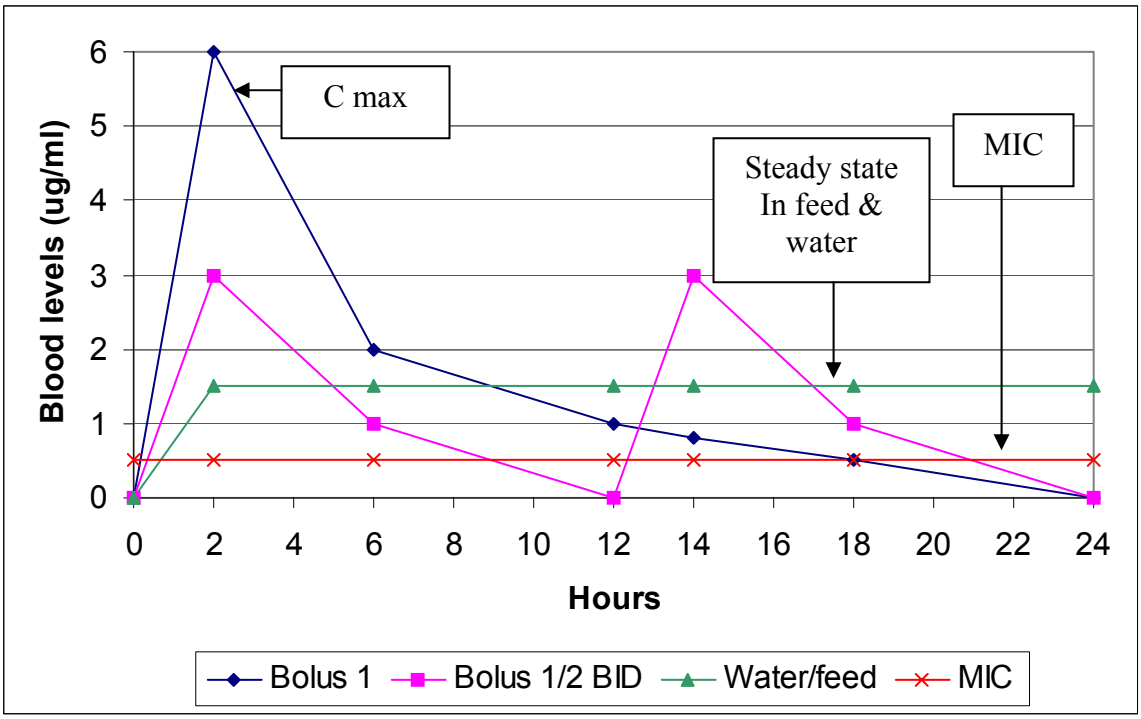

Source: VMD, 2003

The in-feed and oral routes account for 87% of antimicrobials and are very important for pig medicine. Attributing usage to particular species is difficult, as a product may be used in more than one species. However, probably about 40-45% of the total goes into pigs. This is why it is important for pig veterinarians to use these products carefully; to establish a correct diagnosis; remove stressors and other contributory disease-causing factors; check antimicrobial sensitivity; select the right dose rate for the age and type of animal and apply basic PK/PD principles, so improving therapeutic choice and success.

**Pharmacokinetics/Pharmacodynamics**

Classically, pharmacokinetics look at the levels of a drug in the blood/plasma after administration, either as an oral bolus or after an injection (see Graph 3).

**Graph 3 - Typical pharmacokinetics graph**

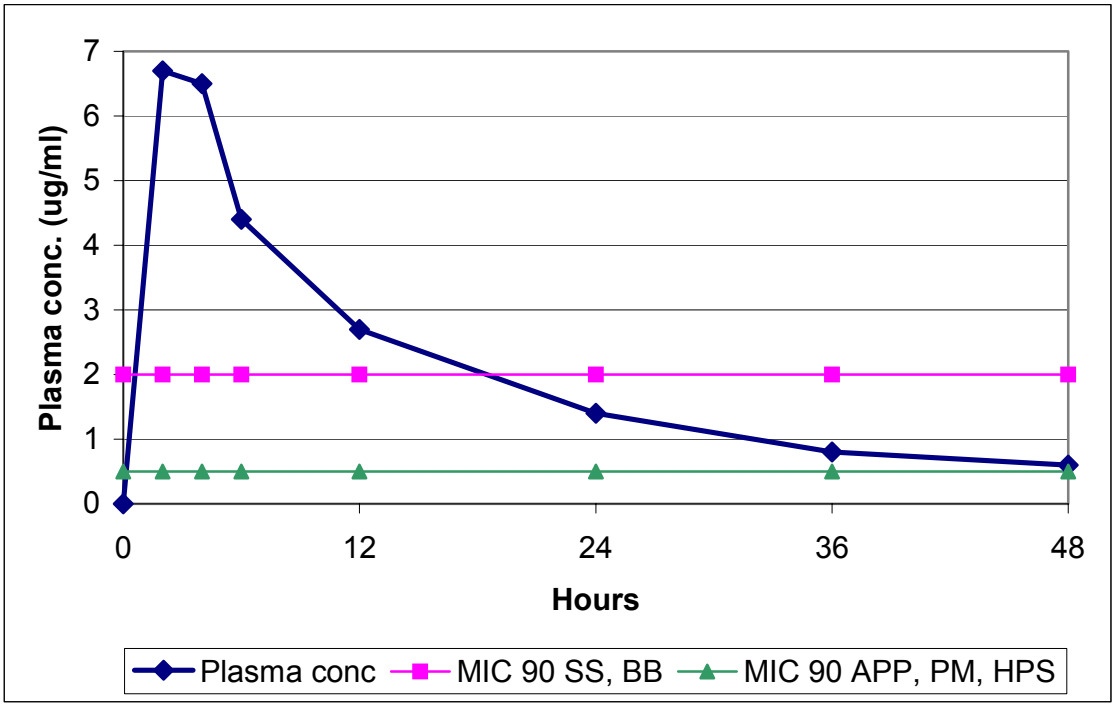



The C max is the maximum concentration reached at a certain time (T max). Ideally, the level of the antibiotic will exceed the minimal inhibitory concentration (MIC) of the organism for a certain time, either to enable the antibiotic to kill it (bactericidal activity) or inhibit it (bacteriostatic action), while the immune cells engulf and kill it. Sometimes a single dose will last for almost 24 hours above the MIC; sometimes two doses or more need to be administered. Some products exert a post-antibiotic effect (PAE), where the activity of the antibiotic continues to have an inhibitory effect after the plasma level has fallen below the MIC and can last for usually 2-4 hours. It also helps to determine dosage intervals. In the case of water or feed administration, a lower C max is reached; but a flatter level, almost a 'steady state,' is achieved over a 24-hour period. A high C max is good for bactericidal concentration-dependent antibiotics, such as the fluoroquinolones and aminoglycosides; but time above the MIC is more important for the bacteriostatic antibiotics like the tetracyclines, macrolides, pleuromutilins and lincosamides, which are commonly used orally in pig medicine. Penicillins are bactericidal; but are also time dependent. Feed passes down the intestinal tract of a pig quite slowly (36-48 hours), so is a good vehicle for applying a long treatment time to a gut infection, especially in the large intestine, where it concentrates. Feed can, however, affect bioavailability, especially in liver-metabolised molecules. Water passes down in about half the time, so is better for prompt treatment and it usually has a better bioavailability for systemic infections. The rate of absorption, distribution, metabolism (especially in the liver) and route of excretion, all play an important role in the final

concentration in the blood or target tissue or fluids in which the infection is located.

For example, a recently introduced injectable for pigs, florfenicol (Nuflor – Schering-Plough) has a relatively high C max in plasma and a long excretion time over 48 hours, when administered at 15mg/kg bodyweight. The plasma levels exceed the MIC 90s (90% of isolates tested) for *Actinobacillus pleuropneumoniae, Pasteurella multocida* and *Haemophilus parasuis* for 48 hours; but less than 24 hours for *Streptococcus suis* and *Bordetella bronchiseptica*; so it is only necessary to treat the former every two days, but the latter every day (see Graph 4.)

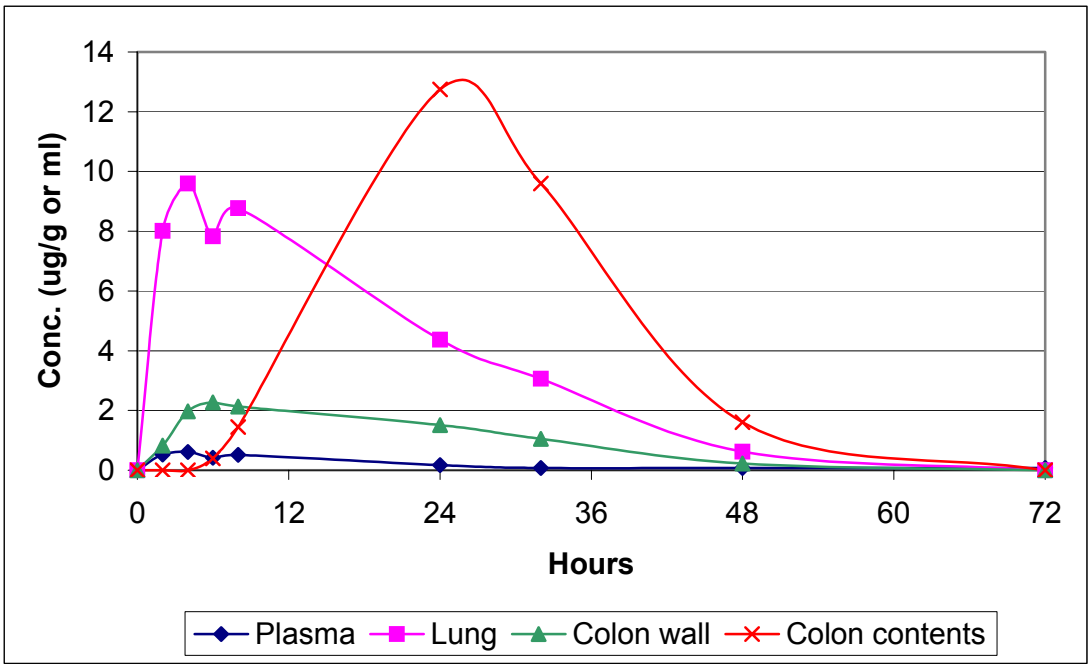
**Graph 4 - The pharmacokinetics of florfenicol injection in relation to various respiratory pathogens' MIC 90s when administered at 15mg/kg bodyweight**




Source: Burch, 2003

Target tissue, or content concentrations, are also very important. Tiamulin (Tiamutin – Novartis) when given by injection has relatively low plasma concentrations, but very high lung concentrations. Its route of excretion is primarily via the liver and bile and thus achieves very high concentrations in the colon contents and wall for over 48 hours following a single injection (see Graph 5.)

**Graph 5 - Concentration of tiamulin in plasma, lung, colon wall and contents following a single injection at 15mg/kg bodyweight**




Source: McKellar, 1993 – unpublished data

Some antimicrobials concentrate in tissues such as the lung, e.g. the macrolides, lincosamides and pleuromutilins, while tilmicosin has been shown to concentrate in lysosomes in macrophages, which is thought to enhance their killing effect against *A. pleuropneumoniae*. Cell penetration is associated with highly lipid-soluble compounds. Some products are poorly absorbed from the gut, such as neomycin, apramycin and spectinomycin; but are very useful for treating infections there, such as *Escherichia coli* and salmonella.

**Oral antimicrobial pharmacokinetics (**www.pigjournal.co.uk**)**

Oral antimicrobial use in pigs is probably the most important and a number of examples are given of products administered by a bolus dose (B), in drinking water (W) and in feed (F) (see Tables 1-3) The data is available on The Pig Journal website.

**Table 1 - Comparative pharmacokinetics of tiamulin, tilmicosin, tylosin, lincomycin and spectinomycin given orally**

| Antimicrobial | Dose rate (mg/kg bwt) | Route B, W, F (ppm) | C max (µg/ml) | Steady state (µg/ml) | Lung conc. (µg/ml) | Colon conc. (µg/ml) |
|---|---|---|---|---|---|---|
| Tiamulin | 10<br>11 | B<br>F220 | 1.03<br><0.3 | -<br><0.3 | -<br>1.99 | -<br>8.05 |
| Tilmicosin | 19.4 | F400 | 0.039 | 0.039 | 1.69<br>7.19 (macrophage) | - |
| Tylosin | 5.5 | F110 | <loq | <loq | <0.05 | 50e |
| Lincomycin | 11 | F220 | 0.14 | 0.14 | 1.13 | 101 |
| Spectinomycin | 2.2 | F44 | - | - | - | 17-50 |

Key: B = bolus; W = water; F = in feed; loq = limit of quantification; e = estimate

The effect of a bolus and in-feed administration demonstrates the effect of feed on absorption of tiamulin. C max figures after a bolus can be misleading if that figure is used to determine sensitivity. The steady state is probably more representative as well as the tissue concentrations, although this is not absolute. This is still an area of active debate (P-L. Toutain – personal communication) with regard to lung concentrations. Sometimes the data is not available. There are comparatively high tiamulin levels in the lung and colon, where it is most useful for treating mycoplasma and brachyspira infections. Tilmicosin and tylosin also have low serum levels, but higher levels in the lung. The macrophage concentration is especially high for tilmicosin. Lincomycin gives a detectable plasma level, a higher lung concentration (8 times) and also a very high colon concentration. Spectinomycin, in contrast, is hardly absorbed from the gut and gives very high concentrations in the colon for *E. coli* and salmonella control.

**Table 2 - Comparative pharmacokinetics of the tetracyclines**

| Antimicrobial | Dose rate (mg/kg bwt) | Route B, W, F(ppm) | C max (µg/ml) | Steady state (µg/ml) | Lung conc. (µg/ml) | Bioavailability (%) |
|---|---|---|---|---|---|---|
| Chlortetracycline | 18 | F364 | 0.35 | 0.25 | 0.55 | <20 |
| Oxytetracycline | 18 | F364 | 0.2 | 0.08 | 0.1 | <5 |
| Tetracycline | 46.6 | W330 | 0.8 | - | - | <20 |
| Doxycycline | 13.3 | F250 | 1.5 | - | 1.7 | <100 |

Chlortetracycline usually gives higher plasma and lung concentrations than oxytetracycline for an equivalent dose in pigs, as its bioavailability is much higher. Tetracycline is similar in bioavailability to chlortetracycline, whereas doxycycline has a very high bioavailability. Plasma and lung concentrations are relatively similar, unlike the former group.

**Table 3 - Comparative pharmacokinetics of penicillin V, amoxycillin, trimethoprim and sulphadiazine**

| Antimicrobial | Dose rate (mg/kg bwt) | Route B, W, F(ppm) | C max (µg/ml) | Bioavailability (%) |
|---|---|---|---|---|
| Penicillin V | 13 | B | 3.39 | 41 |
| Amoxycillin | 20 | B | 6.76 | 39 |
| Trimethoprim | 8.3 | B | 1.9 | 90 |
| Sulphadiazine | 39.1 | B | 32 | 89 |

Penicillin V and amoxycillin are moderately absorbed; but trimethoprim and sulphadiazine are very well absorbed with sulphadiazine giving very high plasma levels. Feed does not impact their bioavailability significantly, presumably because they are excreted via the kidneys rather than the liver.

**Relating pharmacokinetics to antimicrobial sensitivity and product choice**

The use of the product's PK can help with decisions about which product to use. Where is the organism, in the gut or in the body? Does the antimicrobial penetrate that organ and at what level and how does it relate to the MIC of the organism, or has a 'breakpoint' been established? What is the chance of susceptibility - 90% good, 50% moderate or below 50%? This is usually based on the history of the farm initially. The route and dose are also important and this often depends on the number of animals treated and the type, whether sow or piglet (usually individual treatment) or grower/fattener, which usually involves larger numbers and an approach via water or feed. Cost/effect (C/E) is the other balance, with cost being a major driver. Hence the wide use of in-feed chlortetracycline, which has a very good C/E ratio.

**Determining antimicrobial susceptibility**

Antimicrobial disc diffusion test on agar plates (Kirby-Bauer) is the cheapest, quickest and easiest test and is most commonly carried out. It is not the most accurate; but gives an early indication of whether an organism is sensitive, intermediate or resistant based on the zone of inhibition around the disc. Measuring the zone and relating it to known standards for that antibiotic and that particular organism's MIC can improve its accuracy. The National Committee for Clinical Laboratory Standards (NCCLS) guidelines (www.nccls.org) have tried to

standardise the methods used and determine their 'breakpoints' for bacteria based on MICs and efficacy trial work. However, it does not necessarily take into account route of administration e.g. injection and in feed, which can have a very different PK and efficacy consequence.

Broth dilution tests to determine MICs are currently more accurate and useful and the MIC 50, MIC 90 and range can be determined if sufficient numbers of organisms are tested. These can be related to the anticipated PK values and a determination of likely success can be estimated.

**Common porcine pathogenic bacterial isolates and their susceptibilities**

The Veterinary Laboratories Agency (VLA) currently collate and produce data on antimicrobial sensitivity under their surveillance role for the Department for Environment, Food and Rural Affairs (DEFRA). It is based on cases submitted to the VLA centres around the country and may represent a worse case scenario as cases may have received prior antimicrobial therapy. They report their data as sensitivity using the disc diffusion technique and a uniform cut-off point of 13mm diameter zone is used to discriminate between sensitive and resistant strains.

**Table 4 - Antimicrobial resistance (%) of various porcine pathogens in the UK in 2001**

| Antimicrobial | *E. coli* | Salmonella | *P. multocida* | *A. pleuropneumoniae* | *S. suis* |
|---|---|---|---|---|---|
| Ampicillin 10µg | 53 | 54 | 3 | 6 | 0 |
| Tetracycline 10µg | 85 | 83 | 15 | 40 | 65 |
| Neomycin 10µg | 16 | 6 | 20 | 68 | - |
| Apramycin 15µg | 14 | 6 | 8 | 70 | - |
| TMP/S 25µg | 52 | 54 | 9 | 14 | 4 |
| Enrofloxacin 5µg | 5 | 8* | 0 | 0 | - |
| Streptomycin 25µg | - | 42 | - | - | - |
| Sulphonamide 300µg | - | 74 | - | - | |
| Penicillin 10iu | - | - | - | - | 0 |
| Ceftiofur 30µg | - | - | - | 0 | 0 |

Source: VLA, 2002. Key: * = nalidixic acid

In general, this is useful data for surveillance and monitoring trends in resistance development. It highlights that there is resistance development to

tetracyclines and trimethoprim/sulphas, which are two of the most widely used compounds in pig medicine, by *E. coli* and salmonella (mainly *Salmonella enterica* Typhimurium in the pig). It would be more useful to report penicillin, streptomycin (the most widely used injectable combination), ceftiofur and now the newer florfenicol susceptibilities to *P. multocida* and *A. pleuropneumoniae* than to neomycin and apramycin, as these are more likely to be used than the latter.

In general, the MIC of the organism is going to be more helpful than simple sensitivity/resistance testing, especially in relation to PK levels. In the following tables, the MIC data for a variety of porcine pathogens can be seen.

**Table 5 – Minimal inhibitory concentrations (MICs) (µg/ml) of various antimicrobials against 76 field isolates of *B. hyodysenteriae* in Australia**

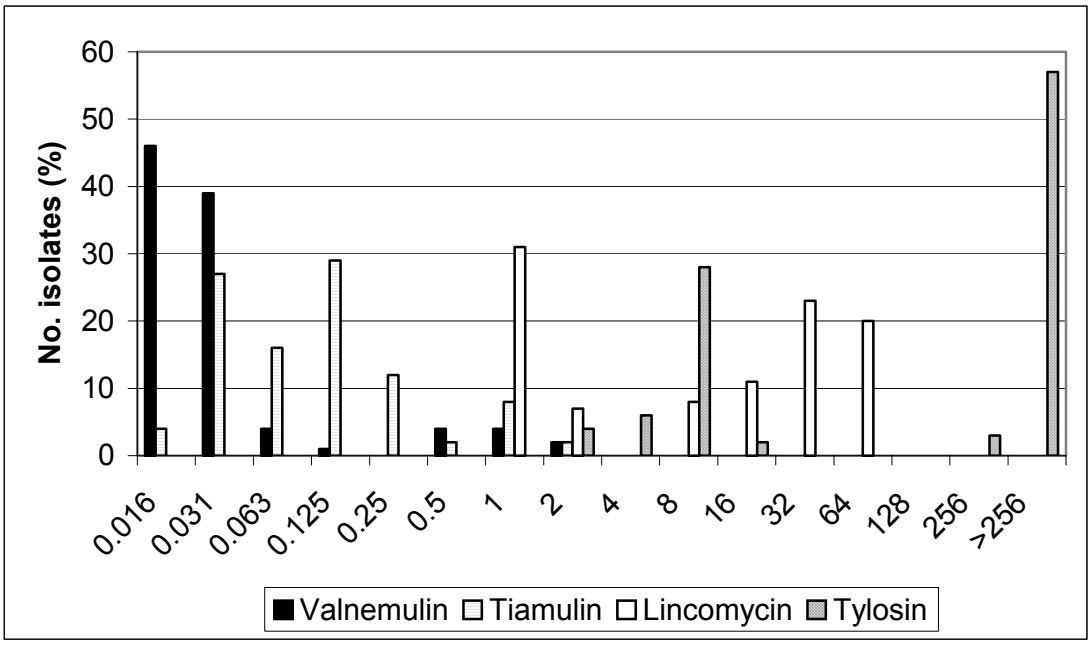
| Antibiotic | MIC 50% | MIC 90% | Range | Estimated breakpoints |
|---|---|---|---|---|
| Econor | 0.031 | 0.5 | ≤0.016 – 2.0 | >4 |
| Tiamutin | 0.125 | 1.0 | ≤0.016 – 2.0 | >4 |
| Lincomycin | 16 | 64 | ≤1 – 64 | >35 |
| Tylosin | >256 | >256 | ≤2 - >256 | >50 |

Source: Karlsson *et al*, 2002

The pleuromutilins are generally highly active against *B. hyodysenteriae*, although there is some resistance to lincomycin and the majority are resistant to tylosin

In Graph 6, percentage sensitive at different concentrations are shown and the various resistance patterns are demonstrated.

**Graph 6 - MIC (µg/ml) patterns for various antimicrobials against *B. hyodysenteriae***




Source: Karlsson *et al*, 2002

This is very helpful if there are large numbers of isolates. There is a shift to the left with valnemulin, which generally appears to be most active against *B. hyodysenteriae.* Tiamulin is less sensitive, but is still below the estimated breakpoint. Lincomycin straddles the breakpoint so some strains are likely to be tolerant/resistant, whereas tylosin has two peaks, one in the sensitive range and a distinct group of completely resistant strains on the right.

For *B. pilosicoli*, a similar pattern exists (see Table 6)

**Table 6 - Susceptibility (MIC 90% µg/ml) of *B. pilosicoli* to various antimicrobials**

| Antimicrobial | Denmark | Czech Republic | USA |
|---|---|---|---|
| Valnemulin | 0.0156 | - | 0.5 |
| Tiamulin | 0.0625 | 2 | 1 |
| Lincomycin | 128 | 64 | 64 |
| Tylosin | >128 | >128 | >512 |
| Carbadox | - | - | 0.06 |
| Salinomycin | - | 1 | - |

Sources: Moller *et al*, 1996; Cizek *et al*, 1998; Kinyon *et al*, 2002

Consistently, the pleuromutilins are more active against *B. pilosicoli* than lincomycin and tylosin, but carbadox (still used in the US) is very active and salinomycin also has some reported activity.

*Lawsonia intracellularis* needs to be grown in cell cultures and an ingenious method for testing its susceptibility to antimicrobials was described by McOrist *et al* (1995). The organisms were grown in the presence of the antimicrobials in the culture media and the percentage inhibition of cell damage, set at 99%, was used to determine the intracellular MIC. Ileitis control has correlated well with antimicrobials levels found in the small intestine and ileum, in particular, and the percentage inhibition shown *in vitro*.

**Table 7 - Susceptibility (intracellular MIC µg/ml) of *L. intracellularis* to various antimicrobials**

| Antimicrobial | MIC |
|---|---|
| Chlortetracycline | 1 |
| Valnemulin | 2 |
| Tiamulin | 4 |
| Tylosin | 64 |
| Tilmicosin | 2 |
| Lincomycin | 32 |
| Spectinomycin | 32 |
| Enrofloxacin | 8 |

Source: McOrist *et al*, 1995

With regard to respiratory pathogens, a recent report looked at *Mycoplasma hyopneumoniae* in Thailand (Thongkamkoon *et al*, 2002)

**Table 8 - Susceptibility (MICs µg/ml) of *M. hyopneumoniae* to various antimicrobials**

| Antimicrobial | MIC 50 | MIC 90 | Range |
|---|---|---|---|
| Chlortetracycline | 0.39 | 1.56 | <0.024-3.1 |
| Josamycin | 0.048 | 0.097 | <0.006-0.2 |
| Lincomycin | 0.048 | 0.097 | <0.006-0.4 |
| Oxytetracycline | 0.195 | 0.39 | <0.024-0.78 |
| Tilmicosin | 0.39 | 1.56 | <0.024-3.1 |
| Tiamulin | 0.006 | 0.048 | <0.006-0.1 |
| Valnemulin | <0.006 | <0.006 | <0.006 |

Source: Thongkamkoon *et al*, 2002

The level of resistance to *M. hyopneumoniae* is surprisingly low around the world. Some isolates may be tolerant to chlortetracycline administered via the feed; but the majority would appear to be very susceptible.

*A. pleuropneumoniae* can be one of the more difficult organisms to treat and its susceptibility is more variable. Injectable products are more commonly required. A useful study of 108 isolates was reported by Barigazzi *et al* (1996a) from Italy.

**Table 9 - Susceptibility (MIC µg/ml) of *A. pleuropneumoniae* to various antimicrobials**

| Antimicrobial | MIC 50 | MIC 90 | Range |
|---|---|---|---|
| Amoxicillin | 0.12 | 16 | <0.03->32 |
| Ceftiofur | <0.03 | <0.03 | <0.03-1 |
| Enrofloxacin | <0.03 | 0.06 | <0.03-2 |
| Florfenicol | 0.5 | 1 | 0.25-2 |
| Oxytetracycline | >32 | >32 | 0.25->32 |
| Pen/strep | 0.25 | 16 | 0.03->32 |
| Tiamulin | 16 | 16 | <0.03->32 |
| Tilmicosin | 4 | 8 | 0.06->32 |
| TMP/S | <0.03 | 0.12 | <0.03-8 |

Source: Barigazzi *et al*, 1996a

There is a high level of resistance to oxytetracycline and some to amoxycillin and penicillin/streptomycin. Trimethoprim/sulpha still looks effective. Tiamulin has a very high MIC; but it has been shown to be effective following drinking water and injectable therapy, though not in feed. This relates to the high levels found in the lung; but the precise mode of action has not been determined, unlike tilmicosin. The main injectable antimicrobials ceftiofur, enrofloxacin and florfenicol look very active.

*H. parasuis* is a difficult organism to isolate and sensitivity data is limited. Trigo *et al* (1996) reported on the susceptibility of 124 isolates from the US using the disc diffusion technique; the majority of bacteria were sensitive.

**Table 10 - Susceptibility (MIC µg/ml) of *H. parasuis* to various antimicrobials**

| Antimicrobial | Sensitive (%) |
|---|---|
| Ampicillin 10µg | 100 |
| Ceftiofur 30µg | 98 |
| Penicillin 10µg | 98 |
| Gentamicin 10iu | 96 |
| Trimethoprim/sulpha 5µg | 94 |
| Amikacin 30µg | 94 |
| Tetracyclines 30µg | 85 |
| Clindamycin 2µg | 60 |

Source: Trigo *et al*, 1996

*S. suis* type 2 susceptibility was described by Barigazzi *et al* (1996b) on 42 field isolates from Italy (see Table 11.)

**Table 11 - Susceptibility (MIC µg/ml) of *S. suis* to various antimicrobials**

| Antimicrobial | MIC 50 | MIC 90 | Range |
|---|---|---|---|
| Penicillin/strep | <0.03 | <0.03 | <0.03 |
| Amoxycillin | <0.03 | <0.03 | <0.03 |
| Ceftiofur | <0.03 | <0.03 | <0.03 |
| Cephalexin | 0.06 | 0.06 | <0.03-0.12 |
| Enrofloxacin | 0.5 | 0.5 | 0.12-0.5 |
| Florfenicol | 1 | 2 | 0.5-4 |
| Oxytetracycline | 32 | >32 | 0.12->32 |
| Sulfamethazine | >32 | >32 | >32 |
| Trimethoprim/sulpha | 0.25 | 0.25 | <0.03-0.5 |
| Tiamulin | 1 | 1 | <0.03-2 |
| Tilmicosin | >32 | >32 | 4->32 |

Source: Barrigazzi *et al*, 1996b

The penicillins and cephalosporins are very active against *S. suis*, as are enrofloxacin and trimethoprim/sulpha. Minimal inhibitory concentrations (MICs) are very high for sulfamethazine alone, oxytetracycline and also tilmicosin.

## Conclusions

The use of therapeutic antimicrobials in pigs is very important as they are estimated to be the largest consumer in the UK as a single species. This puts a large responsibility on pig veterinarians, producers and processors to ensure the meat produced is free as possible from antimicrobial residues, resistant zoonotic bacteria and resistant bacteria, which may transfer resistance to human bacteria.

The use of pharmacokinetics and pharmacodynamics is generally very helpful in determining the choice of antimicrobial and dose that should be used; but other factors often influence this, with cost being the major one.

Antimicrobial resistance/sensitivity tests using disc diffusion are useful, especially for surveillance and monitoring resistance trends, but are limited when it comes to antimicrobial use and probably overestimate the activity. Minimal inhibitory concentrations (MICs), although more costly, do help give a better expectation of success when related to antimicrobial concentrations in plasma and tissues; although this is not always directly related.

There is resistance in the field, especially in *E.coli*, salmonella (mainly *S.* Typhimurium), *A. pleuropneumoniae* and *B. hyodysenteriae*; but, in spite of usage over many decades, the level of resistance clinically is generally tolerable in the UK. Opportunities to control resistance should be explored though, e.g. by improved management, biosecurity, hygiene, operating all-in all-out systems and use of vaccines. It is also fortunate that a number of new compounds and formulations are expected to be developed in this decade and ease the load of the existing products.

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