Feedlot Session II

"Practical Feedyard Pharmacology"

Moderator— Steve Lewis

Use of *In Vitro* Susceptibility Data to Direct Antibacterial Therapy of Bovine Respiratory Disease

Cyril R. Clarke, *BVSc, PhD*
Department of Physiological Sciences
College of Veterinary Medicine
Oklahoma State University
Stillwater, OK 74078

Abstract

***In vitro* susceptibility assays, such as the agar diffusion technique and determination of minimum inhibitory concentrations (MIC), can be used to predict antibacterial activity of a drug against specific bacteria isolated from cattle suffering from bovine respiratory disease. Caution should be exercised in interpreting these data because they do not reflect the contribution of the host defenses to antibacterial efficacy nor do they take into account the effects of inflammatory responses and tissue damage on drug activity. Furthermore, the relevance of pharmacokinetic data used to classify the susceptibility of the isolate should be closely examined. Used appropriately, MIC data provided a more quantitative assessment of activity than the agar diffusion technique and can be used together with relevant kinetic data to guide selection of antibacterial agents and dosage regimens.**

Generally, selection of appropriate drugs and determination of dosage regimens is based on the quantitative relationship between drug concentrations and specific *in vivo* effects on animal target tissues. Referred to as the dose response curve, this relationship provides an objective guide to clinical use of therapeutic agents. Unfortunately, this approach is not appropriate for antibacterial agents because these drugs are administered without any intended direct effect on the host animal. Instead, a dose response relationship must be established for the offending bacterial pathogen. This is usually accomplished using *in vitro* assays, which assess the effects of different concentrations of antibacterial agents on viability and growth of bacterial isolates. These *in vitro* responses are then related to serum and tissue concentrations attained by prescribed doses.

Types of *in vitro* susceptibility assays

The most commonly used method of measuring *in vitro* antibacterial susceptibility is the agar diffusion technique, the interpretation of which depends on the size of a zone of inhibition produced when the bacterial isolate is cultured in the presence of a filter paper disk impregnated with a standard concentration of the same antibacterial agent.[1] Based on the relationship between serum concentration attained after administration of a prescribed dose of a specific antibacterial agent to humans and the standard concentration of the same antibacterial agent in the filter paper disk, bacterial isolates are classified as being susceptible, resistant, or intermediate (moderately susceptible).

Another common estimate of *in vitro* antibacterial susceptibility is the minimum inhibitory concentration (MIC), which is determined by incubating a standard inoculum of the bacterial isolate with serial dilutions of the antibacterial being tested. The lowest concentration of antibacterial agent that inhibits growth is defined as the MIC. Recent use of microtitration trays containing dehydrated antibacterial agents has greatly facilitated routine MIC determination and the availability of MIC data.[2] Although a MIC does not directly predict efficacy in the diseased animal, when compared with pharmacokinetic data "breakpoints" can be identified, which allow classification of isolates into susceptible, resistant, or intermediate groups.

Assumptions and Problems associated with use of *in vitro* susceptibility data

In vitro susceptibility assays, such as the agar diffusion technique and determination of MIC, are conducted in the absence of host defenses and, therefore, may underestimate *in vivo* efficacy. This is particularly true of anitbacterial agents which usually achieve bacteriostatic rather than bactericidal concentrations in sites of infection. Such agents merely inhibit bacterial growth and rely on immune mechanisms to eradicate the infection. Conversely, antibacterial agents judged to be effective *in vitro* may be inactivated in the animal by cultural conditions resulting from inflammatory processes and tissue damage. Conditions responsible for antibacterial inactivation include binding of drug to plasma and acute phase proteins such as albumin and α_1-acid glycoprotein, changes in pH that alter the diffusion of drug into infected tissues and bacteria, and accumulation of cellular debris that may serve as a source of metabolic intermediates negating the mechanism of action of the drug.[3]

An assumption underlying classification of bacterial isolates as being susceptible, resistant, or intermediate is that there is a direct correlation between serum concentration and *in vivo* efficacy. However, lung concentrations may be substantially higher or lower than corresponding serum concentrations. These possible disparities are particularly relevant to subclinical and chronic cases of bovine respiratory disease because abscessation and consolidation of lung tissue may present barriers to drug diffusion. Furthermore, many antibacterial agents are known to exert deleterious effects on bacteria after concentrations have decreased below MIC.[4] These "postantibiotic effects" are associated with loss of bacterial virulence and enhanced ability of host defenses.

Considering that susceptibility classifications determined using the agar diffusion technique are based on achievable serum concentrations in humans, use of these data is discouraged. Determination of MIC provides information of a more quantitative nature that may be used to guide both drug selection and dosage determination.

Practical application of MIC data

The primary goal of antibacterial therapy is to achieve an effective concentration of drug at the site of infection, for a duration sufficient to cause elimination of bacteria. Therefore, any antibacterial agent selected for use against bovine respiratory disease should achieve concentrations in infected lung that exceed the MIC for the relevant isolate for most of the dosage interval. Ideally, this requirement will be satisfied after administration of the approved labeled dose, thus avoiding toxicity and food residue concerns. Although there are numerous mathematical equations[5] that can be used to calculate specific dosage regimens that will achieve satisfactory tissue concentrations, these are complex and are probably not of practical use in the feedlot environment. Consequently, predicted maximum concentrations of antibacterial agents likely to be achieved in lung tissue after administration of approved doses have been calculated using pharmacokinetic parameters[3] and estimates of lung penetration[6] (Table 1). It is suggested that the antibacterial agent selected must, at least, achieve lung concentrations that exceed MIC. Bacteriostatic agents such as the sulfonamides probably need to be maintained above MIC for the full dosage interval. Depending on the potential for toxicity and regulatory restrictions, doses that exceed the labeled dose may be appropriate when the approved doses are not likely to achieve satisfactory lung concentrations and there are no alternative approved drugs. Appropriate higher doses may be estimated using relevant MICs and dosage calculation equations described elsewhere.[5]

Table 1. Maximum serum concentrations ($C_{(max)}$), minimum serum concentrations at the end of dosage intervals ($C_{(min)}$), and maximum lung concentrations ([Lung]) likely to be achieved after administration of recommended doses of antibacterial agents.

DRUG	APPROVED/RECOMMENDED DOSAGE (INTERVAL)	$C_{(max)}$[ε] (μg/ml)	$C_{(min)}$[ε] (μg/ml)	[Lung] (μg/ml)
Penicillin G	Procaine penicillin G + benzathine penicillin G: 8818 IU/kg SC (48 hours)	0.59	0.01	0.2
	Procaine penicillin G: 6614 IU/kg IM (24 hours)	0.62	0.03	0.2
Ampicillin	4.4-11 mg/kg IM (24 hours)	9.40	1.60	2.4
Amoxycillin	6.6-11 mg/kg SC, IM (24 hours)	5.19	1.36	1.3
Ceftiofur	1-2 mg/kg IM (24 hours)	4.58	0.05	1.4
Erythromycin	15 mg/kg IM (24 hours)[χ]	2.4	0.15	6.0
Lincomycin	11 mg/kg IM (24 hours)[χ]	7.17	0.06	3.6
Oxytetracycline	6.6-11 mg/kg IV, IM (24 hours)			
	Hydrochloride -	4.37	1.17	2.8
	Base -	2.93	1.30	2.0
Spectinomycin	20 mg/kg IM (12 hours)[χ]	52.54	0.05	16.0
Sulfachlorpyridazine	40 mg/kg IV (12 hours)	166.71[φ]	0.13	-
Sulfadimethoxine	55 mg/kg IV, then 27.5 mg/kg IV (24 hours)	297.30[φ]	34.25	148.7[κ]
Sulfadiazine	17.6 mg/kg IV (24 hours)[χδ]	20.65[φ]	0.12	-
Tilmicosin	10 mg/kg SC (1 dose only)	0.64	-	9.78
Trimethoprim	3.5 mg/kg IV (24 hours)[χ]	1.78[φ]	0.01	5.3
Tylosin	17.6 mg/kg IM (12 hours)	1.85	0.42	9.3

[χ]Not approved for use in cattle or extralabel dose
[δ]Combined with trimethoprim
[ε]Using highest approved/recommended dose and dosage interval
[φ]Zero time plasma concentration
[κ]Estimated penetration based on studies conducted in other species

References

1. Bauer AW, Kirby WMM, Sherris JC, *et al.* Antibiotic susceptibility testing by a standardized single disc method. *Am J Clin Path.* 1966; 45:493. 2. Phillips I, Warren C, Waterworth PM. Determination of antibiotic sensitivities by the Sensititre system. *J Clin Path.* 1978; 31:531. 3. Clarke CR, Burrows GE, Ames TR. Therapy of bovine bacterial pneumonia. *Vet Clin N Am: Food Anim Prac.* 1991;7:669. 4. McDonald PJ, Wetherall BL, Pruul H. Postantibiotic leukocyte enhancement: increased susceptibility of bacteria pretreated with antibiotics to activity of leukocytes. *Rev Infect Dis.* 1981; 3:38. 5. Gibaldi M, Perrier D. One-compartment model. In Pharmacokinetics. New York, Marcel Dekker, 1975, p 1.