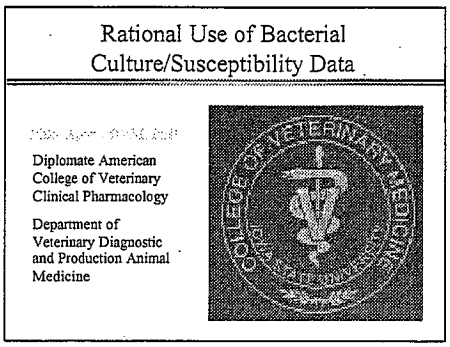
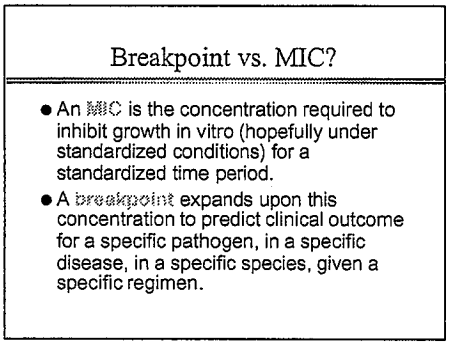
RATIONAL USE OF BACTERIAL CULTURE/SUSCEPTIBILITY DATA

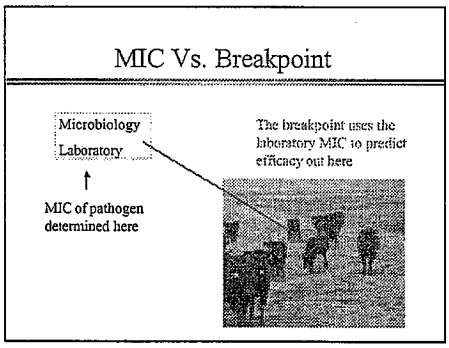
Mike Apley, DVM, PhD, DACVCP



There are some applications of antimicrobials for which the breakpoints used might not be very applicable. As we go through this talk, I was trying to think of an analogy to prepare you for the feelings, to see if you believe whether there is a black box that tells you whether to work or not. When my son, Douglas, was three years old, he spotted a box of ice cream cones in the grocery store, and it had the ice cream cone and the ice cream on top, on the box, and he really wanted those. And we went home and all through supper, that was the topic of conversation. So finally he was ready, and we opened the box and set it down there, and he pulled out that first ice cream cone. His lips started to tremble and he almost broke into tears because there was no ice cream on it. He got ripped off. Well, partway through here, you're going to think I've taken the ice cream away from your cone, because this is not an easy black box. S means yes, R means no. That's the first thing I did in 1996 when I got to Iowa State, was I ran to the back to the food animal clinics and I cornered a senior student, and I said, "What does 'S' mean when you get it back on a susceptibility report?" And they said," Well, that means it will work." And I said, "Job Security".



So the first thing we have to define is breakpoint versus MIC. Now, MIC, minimal inhibitory concentration, is that which is required to inhibit growth *in vitro*, and hopefully under standardized conditions for a standardized time period; 18 to 24 hours. A true MIC is determined by dilution MIC methods. So that is what an MIC is. It is important to understand that the MIC, which is an *in vitro* determination, you can still stick a loop in that, and put the culture in another broth and it will grow again. And so this is different from the min imal bacterial solute concentration. Now a breakpoint expands upon this concentration to predict the clinical outcome. And here is the key part- if it is a validated breakpoint that is for a specific pathogen disease species with a specific regimen. We are going to go through some examples, so a breakpoint is an MIC, but not every MIC is a breakpoint.

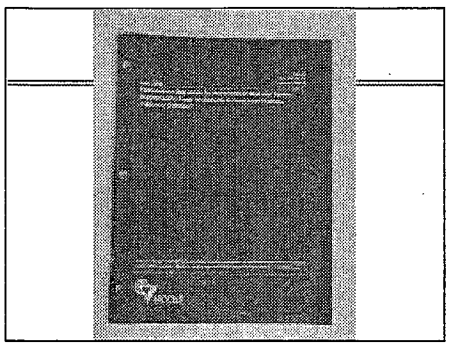


And when you think about breakpoints, it is really kind of presumptuous to work in a lab and have you send in a culture, and then think that by determining the MIC of the pathogen, we can determine efficacy out here. And that is a calf that should have been pulled for respiratory disease yesterday. We send the sample of the cases here, maybe nasopharyngeal or maybe mortalities. Especially ones that are not treated are most valuable- we send them in to the laboratory and you get an S, R, or you and then I start extrapolating out to the herd, which can be a problem.

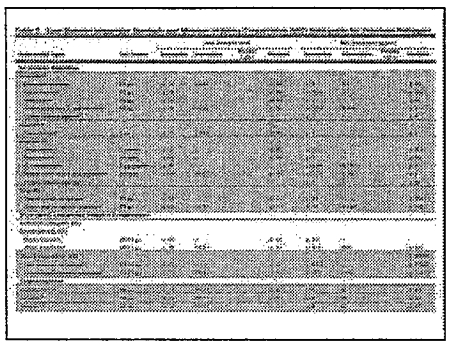
Specifics of a Breakpoint

- Drug
- Pathogen
- Species - age, class
- Disease
- Regimen - dose, route, duration, frequency,duration

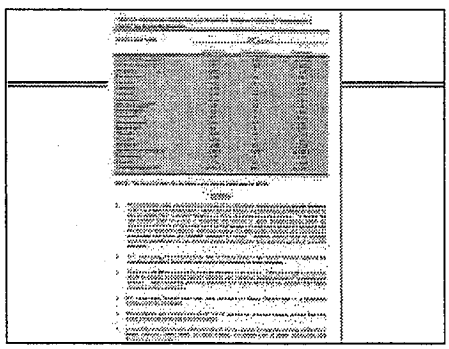
So the first thing we have to define is breakpoint versus MIC. Now, MIC, minimal inhibitory concentration, is that which is required to inhibit growth *in vitro*, and hopefully under standardized conditions for a standardized time period; 18 to 24 hours. A true MIC is determined by dilution MIC methods. So that is what an MIC is. It is important to understand that the MIC, which is an *in vitro* determination, you can still stick a loop in that, and put the culture in another broth and it will grow again. And so this is different from the minimal bacterial solute concentration. Now a breakpoint expands upon this concentration to predict the clinical outcome. And here is the key part- if it is a validated breakpoint that is for a specific pathogen disease species with a specific regimen. We are going to go through some examples, so a breakpoint is an MIC, but not every MIC is a breakpoint.



So this is the N31A, National Committee for Clinical Laboratory Standards, and I've got the main book up there. I have information both in your proceedings and in my abstract, and then in these slides, there is information. If you are performing Kirby-Bauer in your clinic, you should have this document. It has standardized methods, and interpretive criteria.

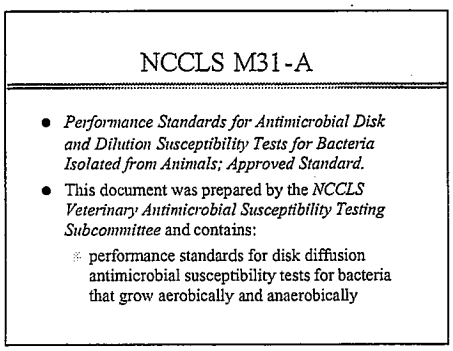


So here is an example of a page with the interpretive criteria, out of the N31A. The ones with dark shades are ones that have been brought over from human medicine, because there are no validated breakpoints for these drugs in veterinary medicine, except for VASTS- Veterinary Antimicrobial Susceptibility Testing Subcommittee of the NCCLS. There are reasonable approximations, or places for us to start. One of the biggest things you need to realize as a practitioner, is which susceptibility results are given to me using a validated breakpoint for my application? And which breakpoints, and therefore, which susceptibility results do I have to very critically evaluate?

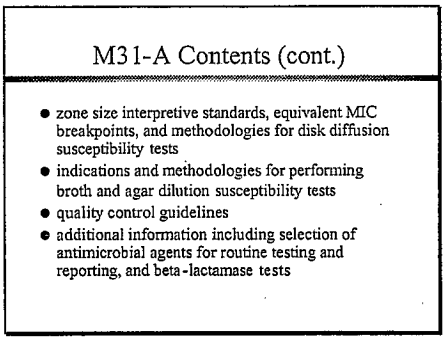


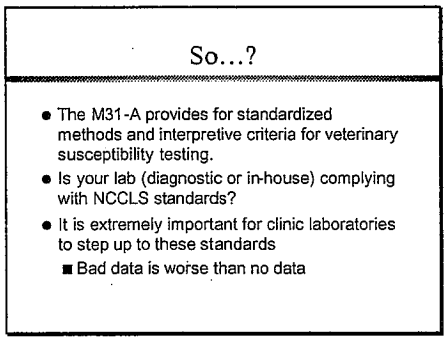
Here is an example of one that does have a veterinary breakpoint, we will list the ones that do have that. There is amoxycillin and clavulanic acid, and procaine, dermal and urinary tract infection application. There are breakpoints for staphylococci. You notice for ampicillin up here at the very top that there is a different set of breakpoints for *Staph, Strep* and *Listeria.* There is quite a range of difference for the *Staph*, 0.25 mcg/ml is the susceptible breakpoint. Of course it is resistance to oxycillin, we discount the ampicillin no matter what, it susceptibility shows zero *Staph*. But for enterobateriasciates, less than 8, that could cause a dramatically different susceptibility result on whether you are using the interpretive zone side related to, they are correlated back to less than or equal to 8, or the zone sizes are correlated back to less than or equal to 0.25. So whichever one you use can make a very dramatic difference in S, I or R. And here are the anaerobic criteria. So the full name for that is Performance Standards for Antimicrobial Disk and Dilution Susceptibility Test for Bacteria Isolated from Animals, and again, this is all of your proceedings, so you can order this. And what it contains are performance standards for disk diffusion tests, for both aerobes and anaerobes. It contains zone size interpreting standards, the MIC breakpoints that are equivalent and then the methodology conducting disk diffusion susceptibility tests. If you run Kirby–Bauer without strictly adhering to standards, and then you standardize the interpretive criteria, I would maintain that you

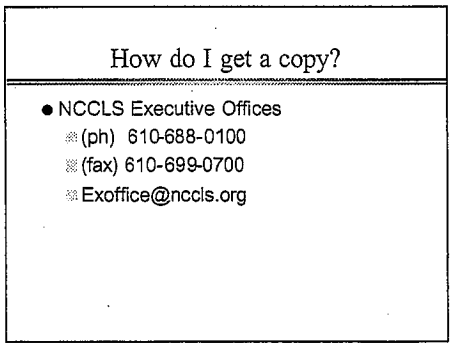
have a case of bad data being worse than no data, because you are applying it back to breakpoints for which you have completely violated the standardized methods for use.



Broth and agar dilution quality control guidelines and then determining different agents for routine testing. So standardized methods and criteria, and again the question about your lab, your diagnostic lab, you need to be clear on which breakpoint they are using, if there are multiple breakpoints available for a drug. And if you call, you will probably have to go through three or four people to find that information. I have great respect for our diagnostic colleague, but if they are looking at S,I or R, and making dose recommendations off just S, I or R, we need to go a whole lot deeper than that. And again, if you are interested in that, I would invite you to come back after break when we go into the support system we are developing, wrestling with. We don't have all of the answers, but at least we are going to point out some holes.







And here is how you get a copy, you can phone or email them. They are about 35.00, for this document.

NCCLS Breakpoints

- Specifically derived for veterinary medicine
  - Bovine respiratory disease - ceftiofur, tilmicosin, florfenicol, enrofloxacin, spectinomycin sulfate
  - Swine respiratory disease - ceftiofur, tilmicosin

So what are the NCCLS breakpoints specifically derived for veterinary medicine. Bovine respiratory disease is often one of the first labels, and one of the biggest markets, so it is often one of the first labels for antimicrobial veterinary use. Ceftiofur, tilmicosin, florfenicol, enrofloxacin, spectinomycin sulfate. And for swine there is ceftiofur and tilmicosin, these have NCCLS validated breakpoints.

NCCLS Breakpoints

- Specifically derived for veterinary medicine (cont.)
  - Others
    - –amoxicillin/clavulanic acid (canine dermal, UTI)
    - –enrofloxacin (canine and feline URI, UTI)
    - –sarafloxacin (poultry)
    - –pirlimycin (bovine mastitis)
    - –penicillin/ novobiocin (bovine mastitis)

Other breakpoints specifically derived for veterinary medicine, again, amoxycillin, clavulanic acid or clavamox, for both dermal and urinary tract infections. Enrofloxacin, for canine and feline upper respiratory and urinary tract. Sarafloxacin, this label has now been withdrawn, because of the notice of opportunity of hearing by the FDA regarding that label. Pirlmycin for bovine mastitis, it is a lincosamide, and penicillin/novobiocin for bovine mastitis. The ones that have actually gone through the NCCLS approval process, these are the ones that I am aware of at this time. There are numerous more that will be brought through the processes. Anyone in this room with all he expertise we have know of any one of them I have left off here? So it takes two slides to go through the veterinary validated breakpoints, and even then they are validated for specific applications. For example, enteric disease; we have become very fond of isolating organisms from an enteric disease, sending in isolates, and then looking for the S's to treat. There are no veterinary validated breakpoints for enteric disease, so I will go through some of my thoughts on that.

NCCLS Breakpoints

- Human breakpoints used in veterinary medicine
  - Beta-lactams - ampicillin,oxacillin, Pen G, ticarcillin, ticarcillin/clavulanic acid, cephalothin, **cefazolin**, cefoxitin, imipenem
  - Aminoglycosides - **amikacin**, **gentamicin**, kanamycin

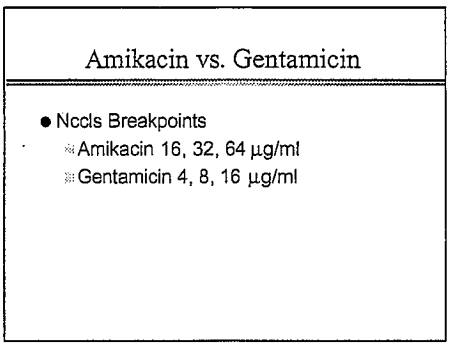
So these are human breakpoints used in veterinary medicine. Your beta-lactams, the ones here in yellow I am going to talk to you about specifically. The ampicillin, the amoxycillin is used off of ampicillin, so the ampicillin is used also to interpret for amoxycillin, *in vitro*, mg potency is very similar, *in vivo* pharmacokinetics orally can be very different, for example in dogs, you have roughly 60 % oral bioavailability for amoxy and about 30% from ampicillin. So there are differences there. All of your aminoglycosides breakpoints are human derived.

Cefazolin vs. Ceftiofur

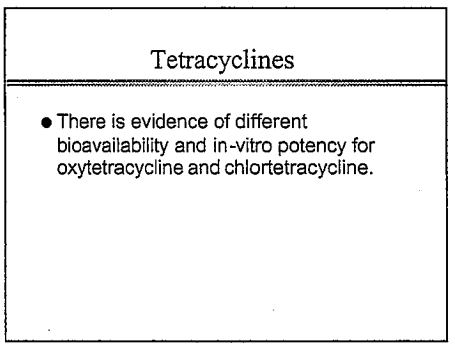
- First generation vs. third generation
- Breakpoints used in food animal panels
  - Ceftiofur – 2, 4, and 8 µg/ml
  - Cephalothin – 8, 16, 32 µg/ml

So here is an example of some things that have been around. In the last several years, there got to be the practice of compounding generic human cefazolin which is a first generation cefalosporin and selling that as a 'replacement' for ceftiofur, which is a third generation cephalosporins The center for veterinary medicine has put out a specific communication on that saying that these are not equal and this is an illegal practice because we are compounding a copy and it is not even a true copy of a true product. The people would tell me, 'Hey, we have gram negative activity for cefazolin.' If it showing susceptible it is *Pastuerella*. First of all, I have never seen the pharmacokinetics of cefazolin adequately described in cattle, and number two, the breakpoints for ceftiofur are 2, 4 and 8 mcg/ml, that means for it to show susceptible, to be reported as susceptible for an isolate, there can be no growth in a 2 mcg/ml, or a zone size that is correlated back to that, it has to be demonstrated on a Kirby-Bauer. Cephalothin, which would be used as a first generation indicator for cephalozolin goes at 8 16 and 32 mcg/ml, so it got a two-dilution benefit of the doubt. But very few people realize that, that might be one of the reasons it even

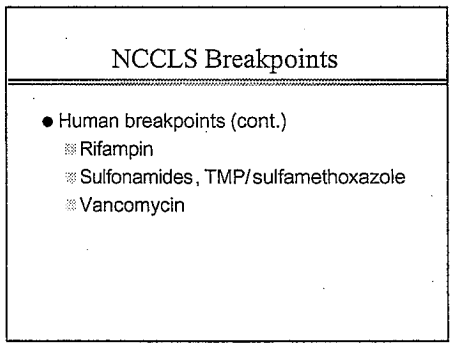
shows gram-negative activity, is because you are getting very high concentration on most of the food animal panels to inhibit the growth. This is just one demonstration of knowing when it is apples versus oranges. If you have the NCCLS N-31 A, you can look this up, and with one of these tables I showed you, you can determine what the breakpoints were for if you were using the Kirby-Bauer method.



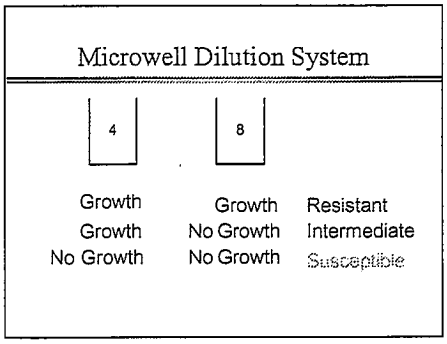
What about amikacin and gentimicin? Well, in some cases, amikacin is actually more refractive to some of the enzymes produced against it, such as the *Pseudomomas*, but also since amikacin in many instances is less toxic than gentimicin, it has a higher dosing regimen. You are all familiar with the dose of amikacin versus gentimicin. To correspond to that, amikacin has susceptible breakpoint of 16 and gentimicin has a breakpoint of 4, so again there is a two dilution jump from 4 to 8 to 16 for susceptible amikacin. And the take home there is if you get an S back for amikacin and decide that you will be really safe and use it at a gentimicin dose, you have really just set yourself up for being fooled, because the pharmacokinetics are going to much more closely mimic that what you would see with the typical gentimicin dose, figure you are giving it a 2 dilution benefit of the doubt at the susceptibility breakpoint. Erythromicin, it is a human breakpoint, we have used tetracycline in the past and I will discuss that on the next slide, that is a human breakpoint. Clindimycin of the glycosamides has a human breakpoint as well as florfenicol at the human breakpoint.



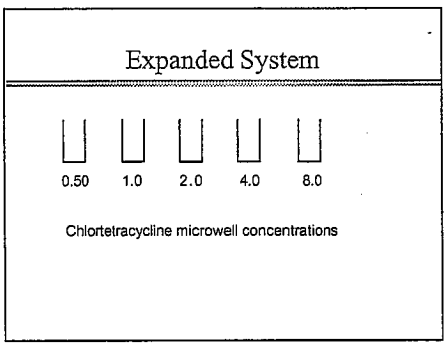
For the tetracyclines, there is now some evidence of different bioavailability and *in vivo* potency for oxytet and chlortet and some isolates in some species of animals enough that at Iowa state as we go to our extended dilution breakpoint system on the serial dilution plates that we are using, oxytet and chlortet, separately and going through extended dilutions other than just relying on tetracycline at 4, 8, 16 mcg/ml, the human breakpoints.



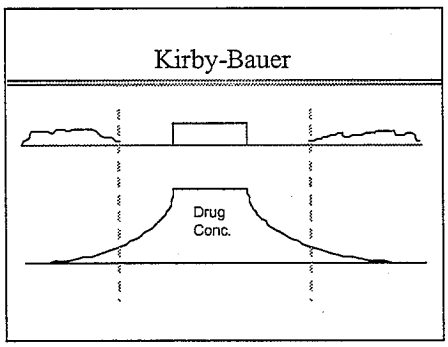
Sulfonamides I mentioned here because these are human derived breakpoints, and I think they are one of the ones that I probably approach with some trepidation about evaluating the breakpoints because site of infection and environment has such an impact on how they function. I tend to look at a sulfonamide breakpoint as really a suggestion.



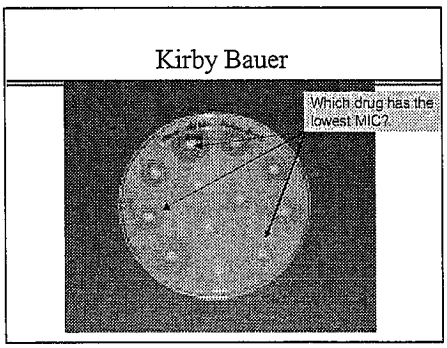
So how does the Microwell dilution system work? A lot of the diagnostic labs use this. There are two wells, and this is the example of the tetracycline human breakpoints that are listed in N31A, 4 and 8, growth in both of these is resistant, and we don't go ahead and check the 16, because resistance is defined as greater than or equal to 16 mcg/ml. So that is all it could test out at if it grows in 8, so for economic reasons, we don't put 16's on the plate to make room for something else. That would be resistant. If the there is growth in 4, but no growth in 8, then that would be called intermediate. Now intermediate is really meant as a warning to you that you are getting to the edge. For a microbiologically derived breakpoint, which we will go through an example of, it is warning you that you are getting to the edge of your population of bacteria that they have characterized. For a PK/PD, pharmacokinetic/pharmacodynamic breakpoint that was set in that manner, it is telling you that you are getting to that edge where you are outside of what you think is a reasonable association. And if there is no growth in either one, it is reported out as susceptible, less than or equal to 4, and as a pharmacologist, I am left using 4 as a worst-case scenario. It may very well be 0.06 mcg/ml, but we don't know, and that is one of the reasons we are going to more extended dilution testing so that we have more of an idea of exactly how well this works.



So there is an example of the expanded system for chlortet, we go down to .5, we still report out S's and I's based on the activity at 4 and 8, but now on the ISU diagnostic lab reports, you might get susceptible at .5 mcg/ml up to this point. We are going to look at some kinetics, one of my favorite slides of oxytetracycline injectable kinetics. We talk about why that for oxytet we go down to .25, well, I am so excited to see that.

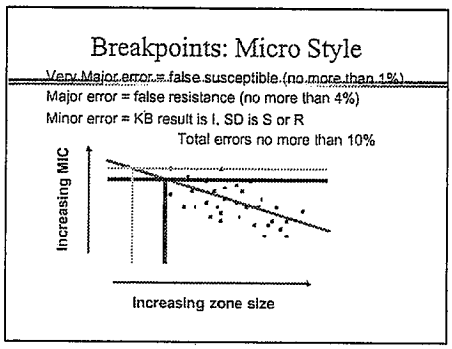


Now, Kirby-Bauer, the way that system works is the disk agar goes on the plate, and all of you have done this in vet school, or you may do it in your clinic, but there is a zone of inhibition around that disk, and the drug concentration falls away from there and when we read it at standardized time after standardized temperature, inoculums, standardized agar type and depth, etc. We correlate that zone of inhibition with an S, R determination or I.

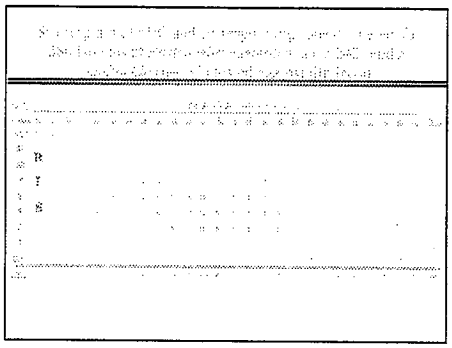


So here is a quiz for you; Which of these drugs has the lowest MIC? And we will call this one A, this one B, this one C. So if you know that zone size diameter is proportional, or inversely proportional to the concentration of a drug that inhibits that organism, is it A, B or C? How many vote for A? How many would say that there is just insufficient data here? Very good. I always get a few people to volunteer, but that is very important to realize, I have been in clinics where they were going with the biggest zone, and it would just collapse, hopefully we will all be evangelists for that. There are specific interpretive criteria and the reason is that each drug has different diffusion characteristics, so the diffusion characteristics and the concentration required to inhibit growth have an effect on zone diameter. There is a company now where the oil ....., showing this brochure around where they put

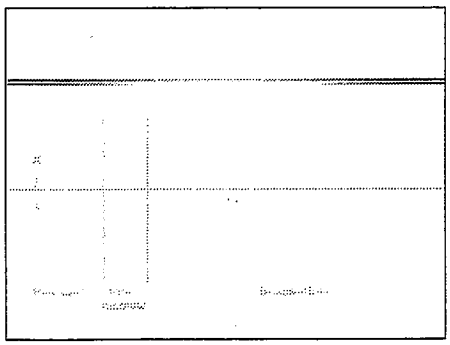
this type of antibiotics against *E. coli* , and they punched a little hole in the middle of the plate, and they dropped their super oil in there, and had a nice four color brochure, comparing zone sizes. We did have a talk about that, but I am not sure that something, obviously that is very bad data. So how do we get to Kirby-Bauer interpretive criteria? How do we get to that? See, at one time, before I pulled the ice cream cone out of the box, I had the assumption that the zone diameter was specifically correlated to a concentration of drug in the model. That is not how it is done.



What they would like to have done is 300 to 500 isolates, graphed this way, and we have increasing zone size with increasing MIC, and hopefully there is a correlation, this is a very stylized ideal correlation showing increasing zone size nicely correlated with increasing MIC, and so you draw in a nice 45 degree line go home, and go to sleep soundly, and everything is nice. And in reality, it is kind of like a pharmacokinetics study; before you run the data through modeling and put it together, report the mean line, it is really nasty. It is really messy, none of the animals read the textbook, and if you actually did the 6 or 8 animals again 30 days later and challenged someone to take the curve and lay it on top and match it to the animal 30 days ago, you would mismatch about half the animals. It is the same way with this, it is kind of messy, and so it really looks more like a blob. But what is done to correlate this back, this is one, the microbiological approach, okay; this is the microbes to the PK/PD approach. There is a technique called error-rate value, and again, from a microbiological approach- before I do that; On the PK/PD model, you can still use this type of technique to try to correlate zone size and MIC. But in this matter, we are going to go ahead with the microbiological approach. And we are going to set error-rate bounds on this using error-rate bounding. Now we are going to set an intermediate line based on dilutional MIC's, and we have done serial dilutions, and intermediate line, the goal is to warn you that you are getting to the edge of the population that you will see a clinical response with. It is not telling you that you may not get clinical response for sure, but they are warning you that you are to the edge of the ones that we are seeing on there.



So that is on dilution, and then they are going to come down and they are going to also cut off the edge of that using a line for your zone size. And then they are going to do the same thing for resistant, telling you that you are way out of bounds. So, there error-rate to that that they try to avoid is first of all, a very major error, they would like no more than 1% of these to show a susceptible by zone size, when in fact, the serial dilution technique which shows it as resistant. So that is a very titered error. A major error would be false resistance, where the zone size shows it as resistant, but the serial dilution would actually classify this as susceptible. And then the minor errors where the Kirby-Bauer results were intermediate, and the serial dilution shows susceptible and resistant and they want the total errors no more than 10%. Now I am actually going to take you through some data from a microbiologically applied a dry breakpoint. David Mc Cleary from Alanco supplied us some stuff for these data to be purposes of illustration, I would like to thank him at this time, and there is also another set further on. The purpose of taking you through this is to impress upon you, the amount of data and effort that has gone into a validated breakpoint for a specific application. So here we have 380 isolates, this graph combines *P. haemolytica* and *P. mulnoelda* and for example, right here, 14 isolates had a zone diameter of 16, and a serial dilution MIC of 8. So you'd say, "Hey, 16 is correlated to 8, well, 32 isolates, 16 was correlated to four and for 67 isolates, 16 was correlated to 2." So you can see, this is like the nice stylized drawing, you can draw the 45 degree line through it, but this is a blob, with a little bit of a ledge up here and a little bit of a ledge down here, we can predict....



Now there are systems out there that work by taking a zone diameter and calculating a precise MIC for you, I am still getting on board with that, but there is work in the literature that maintains that actually the diffusion rate characteristics in Kirby-Bauer results are more consistent than rerunning a serial dilution. I am no expert on that; I will just tell you that controversy is out there. It is common laboratory variation, to run one, an isolate one time and come up with an 8, then have it jump to 16 or to 4. So a one dilution jump on rerun is common to have. So how did they go about this, here are the error-rate bounds they came up with. The breakpoints for tilmicosin in bovine respiratory disease with these pathogens are 8, 16 and 32. Eight susceptible intermediate, 16 to 32 resistant, and then they drew down, as you can see here is the intermediate line and they also drew it down this way for zone diameter. And then here is your resistant. So that is set up around these isolates to specifically guide you for predicting clinical response in respiratory disease. If you apply these interpretive breakpoints to the serial dilution of another organism out of another disease, this has not been done for that, so you are really kind of out on your own. So this is the added importance and comfort you should have in a validated breakpoint.

What's new?

- ISU has been using extended dilution microwell susceptibility testing since October 1999.
- This method gives additional information beyond just S, I, or R.
- So, what does this method do for us?

We have been doing this since October of 1999, and what does the method do for us? First we are going to go through the examples of what we have done.

Expanded Dilution System

Drug	Current	Expanded
Ampicillin	8,16*	0.25 - 32
Apramycin	8,16	4 - 32
Ceftiofur	2, 4	0.5 - 8
CTC	4,8 (TC)	0.5 - 8
Clindamycin	0.25, 0.5*	0.25 – 2

*Ampicillin for enterobacteriacae, Clindamydin for strep, other breakpoints for different indications

Ampicillin- We have done 8 and 16 mcg/ml, particularly being what we have used on many of the panels and the expanded now takes it down to 0.25. After break, when Dr' Langston gets up and goes through some of the modeling we are doing, you will see, especially with the ampicillin trihydrate for the model, why it would be so important to know if the organism is inhibited down to 0.25, rather than just knowing that it was inhibited to 8, for some of our predicted models.

Expanded Dilution System

Drug	Current	Expanded
Enroflxacin	0.25, 2*	0.12 - 2
Erythromycin	0.25,0.5*	4 - 32
Florfenicol	2,4	0.25 - 8
Gentamicin	4,8	1- 8
Neomycin	8	4 – 32

*Erythromycin for strep, enrofloxacin for BRD, other breakpoints for different indications

These are some of the expanded versions we are using. Gentimicin now goes down to 1; florfenicol now goes down to 0.25. So that is a quick run through on where these come from. Other drugs, you can ask the people, the companies to define these findings to you, but some of them were primarily done on the pharmacokinetic/pharmacodynamic equivalent, so it varies how the breakpoints will act.

Expanded Dilution System

Drug	Current	Expanded
OTC	4,8 (TC)	0.25 - 8
Penicillin	0.12,2*	0.12 - 8
Spec (sulfate)	32,64	8 - 64
Sulfachlor	256, 512(R)	32 - 256
Sulfadi	256, 512(R)	32 – 256

*Penicillin for strep, other breakpoints for different organisms

So now we are going to take our newfound knowledge of exactly what the breakpoints are for these different drugs and try to apply it clinically, the next question becomes, pharmacodynamics and kinetics. How should the drug be exposed to the bacteria, and then can we do that in this specific animal? First of all, there are several illusions, and one is that if the reported t 1/2 for oral cephalexin in dogs is 1-½ hours, the illusion is that the elimination half time in a dog on your table is that time. It could be 30 minutes; it could be 10 hours, so there are radical differences in the individual animal that you may be treating. And of course, disease indications may have an effect on that and a lot of tomorrow morning in this room will be talking about the disease effects on paraffin.

Expanded Dilution System

Drug	Current	Expanded
Sulfathi	256,512(R)	32 - 256
Tiamulin	8,16(R)	4 - 32
Tilmicosin	8,16	4 - 32
TMP/Sulfa	2,4(R)	0.5 - 2
Tylosin		2.5-20

Typically we will refer the aminoglycosides and fluoroquinolones as concentration dependant. Fluoroquinolones actually AUC, area under the curve compared to the MIC of the organism and the ratio, and we can argue about whether it should be 125 or 500. Very few practitioners are going go in and say, 'I want to increase the MIC or the area under the curve of this.' But you will get to increase the peak, and when you increase the peak that also drags the area under the curve along with it. In some of the studies I reviewed in fluorquinolones, the peak and the area under the curve were very closely related to predicting. Bacteriostatics, we typically say time above MIC and the beta-lactams, there is information out there now, that for example, for zithromycin and erythromycin, newer generation macrolides and that they may be actually more peak dependant than time above MIC and they are killing diads. So going away from the classic erythromycin model that we are used to. Now what about dosing them to avoid resistance? I don't know much about that.

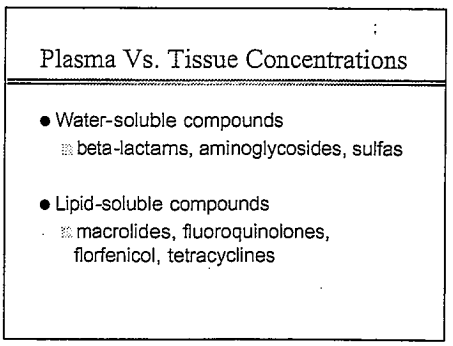
Optimal Dosing Regimens

- "Concentration" dependent:
  - aminoglycosides, fluoroquinolones
- Time above MIC:
  - bacteriostatics, beta-lactams
- Exceptions?

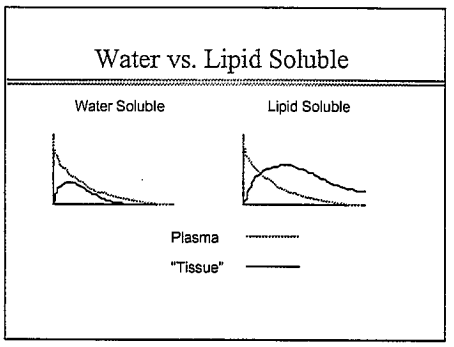
There may be others that do, but I have not been able to find very much really about suppressing resistance. There is now talk in literature about in addition to an MIC and MBC, having a minimal concentration for fluorquinolones, it suppresses resistance. Work in human *E. coli* and *Pseudomonas* isolates *in vitro* would suggest that 10x the MIC is a peak, is a good target. So there is some work out there in fluoroquinolones on avoiding resistance. Interpreted as avoiding selecting for resistant isolates, not a worthy cause of mutation. For aminoglycosides, ideal efficacy ratios have been reported as 8 to 10 times the MIC of the organism, but what about suppressing resistance, I am not really aware of that.

So, what concentrations do we use?

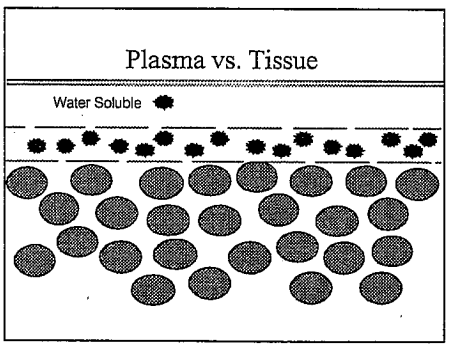
For beta-lactams, I don't know, I always just go back to minimizing exposure, using an appropriate dose for the least amount of time to get it done. So what concentrations are we going to use? Pharmacokinetically, we are going to use some concentrations to compare to the MIC. I would propose to you right now that the people that best understand tissue concentration versus MIC relationships work in marketing departments. They are the ones that I have met that have the clearest understanding of exactly what those relationships mean. Now I have been left off the hook. If you say that at a company meeting, you want to be sure and get out the door before some of the people in the room realize what you said.



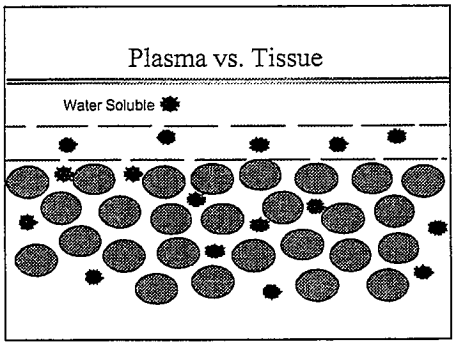
So plasma versus tissue concentrations. What concentrations are we going to typically compare to an MIC. And this is really very important, if we are going to talk about dynamics and kinetics, what concentrations we use. For the water-soluble compounds, beta-lactams and aminoglycosides and sulfas, common practice is to state the serial concentrations, to compare to the MIC of the organism, if you are trying to for example, for the time above MIC for a beta-lactams, if you take ampicillin. For gram-negatives, some of the literature would suggest 90 to 100% of the dosing interval. It would probably be more realistically be closer to 90. For gram-positives, some may design a regimen for 50 or 60 % of the dosing interval over the MIC of the organism. But you will typically stay with serum and we are going to go over some examples in a while. Lipid soluble compounds, macrolides, fluoroquinolones, florfenicol, tetracycline, fluoroquinolones and florfenicol, high tissue concentrations as well as maintaining pretty high serum concentrations. Tetracycline is similar, high serum concentrations plus reasonable tissue concentration. Macrolides, to me are a little trickier. For some of them, we use for example, tilmicosin, there is just very little serum concentration compared to huge major tissue concentrations. So what is going on here?



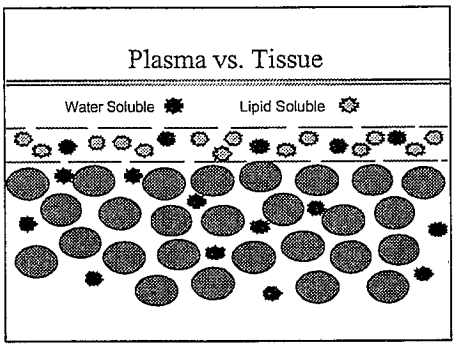
We are familiar with these curves that show the higher plasma or serum concentration versus tissue, and this is a water-soluble relationship and this is a typical lipid-soluble relationship, well, what is up with that?



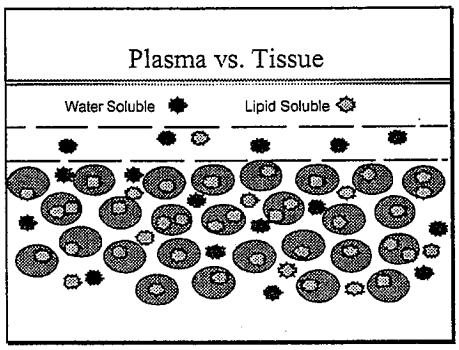
This is tissue with stylized cells and this is the intravenous compartment, now if we have just i.v.'ed a large soluble compound, and now we are going to let it distribute. Our typical concept of this is that the water-soluble compound will tend to stay in the intercellular fluid rather than penetrate into the cells through the lipid membranes. This is primarily been worked out with cell membranes. So when you take out a chunk of tissue, grind it up and extract the drug, and maybe sonicate the cells, you are averaging the concentration of that drug over the intercellular fluid as well as the cellular component, and you are going to tend to make a large soluble compound such as a beta-lactam look like there is hardly any in there, when in fact, you are just taking the concentration that is intercellular and spreading it over areas that the drug doesn't get to.

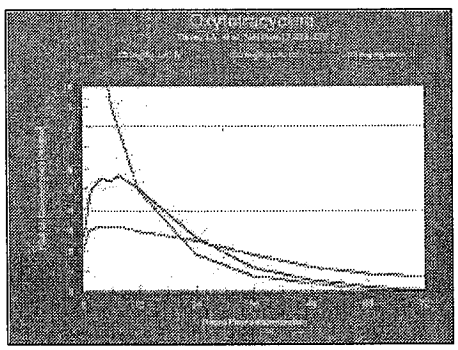


Now what about a higher lipid-soluble drug? Now represented in the green, we are going to let it distribute. You notice that many of those, at least in cells of the immune system seem to be very highly represented in an area with a lot of infection, and we get tremendous concentrations. Some drugs are capable of hitting 1,000 mcg/ml in internal cell concentrations; very, very high *in vitro* exposure. But now we do the same thing in predicting that drug, and instead of biasing for a low concentration, we may actually be biasing toward a very high concentration where the infection actually is. If it is an intercellular infection, there is a lot of the drug located intracellularly. Now this drug, may still be available, but I still have trouble taking a tissue concentration of some of these highly lipid-soluble drugs and going straight across the MIC.



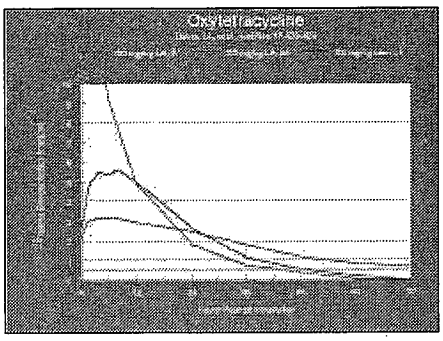
Without having some clinical backup, I don't know. So we take a fluorquinolone in the prostate. We have prostate concentrations and it goes three across that prostatic concentration to an MIC, and we take the same kind of approach to the pharmacokinetic/pharmacodynamic approach as we would as if it was a serum concentration, and I am sure open to be edified in that, but I haven't made that leap yet.



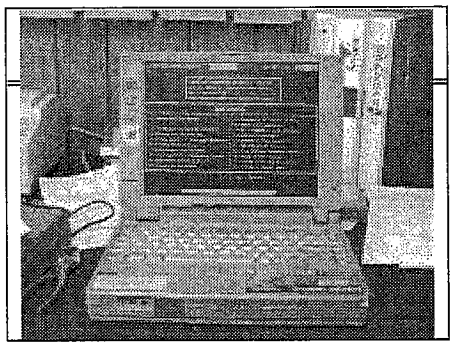


Let's go back to oxytetracycline and actually show how we go about this, and this is my pre-VADS days, where people like Cory and Virginia started asking me a lot of obnoxious questions, about how can we really do this? First thing, these mean data and single dose data, which for a long-acting oxytetracycline here, it would be reasonable to use. But if this is say, an i.m. injection of 100 ml/ml oxytetracycline product here. These are all in the same mls/kgs dose. This is conservative for a long-acting oxytet, because typically, we would see it up around 5 or 6 range for peak. This has actually only got 200 data for oxytet, but say you were dosing this in 24 hours, you would get dose accumulation at steady state and we might actually have a lot better picture once we got repeated doses. But you can see that even if this did peak up here, at the purple line, we would still be wondering out here at 24 to 36 hours what really is the MIC of that organism, and again, this is mean data, single dose. But now if we start testing down to .5 to .25, it gives you a lot more information for producing a reasonable regimen that is going to protect you on out there on organisms for which there is no validated breakpoints, which for oxytet, is all of them. And also, feed and water administration of oxytet, we have studies where they devage the animals one time with 10 mg per pound for the whole daily dose, and you still end up bumping into one-half or .25 mcg/ml, and used in the

field, where they would be taking in small meals or drinks during the day, you would be getting extremely low concentrations in the serum, so if we are going to do pharmacokinetic/dynamic modeling, it would be important to know if it is actually inhibited down to .25.



When in practices, veterinarians get back an S for tetracycline and they say, "Well, should we use an injectable, or should we use a feed?" See, there can be a drastic difference in the pharmacokinetic/dynamic exposure to that organism. Now, hopefully, I can put a little bit of ice cream back in your cone. I actually just kind of dump some of it on the ground in this next part.



I teach pharmacology, all species, to veterinary students, but my clinical practice is still in feedlots, and one of our biggest, neates t toys is real time surveillance, case fatality, morbidity, and mortality. Usually yards that have a 3 to 4,000 head one time capacity we'll start doing this. I work in yards with 100,000 head that when we made our bimonthly visit, the first thing we did was go in and start punching up treatments, and in the fall, in the last 30 days, we may have had 1500 calves treated. And so, more of the susceptibility testing we were relying on what type of clinical response we were getting to a drug regimen. So how does the concept of susceptibility weigh out against computer records?

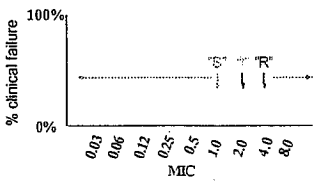
Could there be clinical failures with an "S" isolate?

Or, "since there was a clinical failure with an S isolate, the breakpoint should be lowered"..

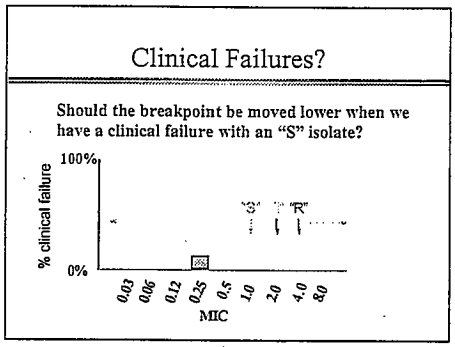
So the first question is," Could there be clinical failures with an S isolate?" And this is something that has been stated, and I spend too much time, actually, in the resistance issues and food animal antimicrobial issue arena, there is many others in the room that do also, and many of them are shaking there heads and glowering. But the deal was, if you have a clinical failure with an S isolate, then the breakpoint should be lowered to where the isolate was that you had the clinical failure. So let's examine that type of concept. It is just one of the many concepts I have failed to come along on, which doesn't mean it is wrong.

Clinical Failures?

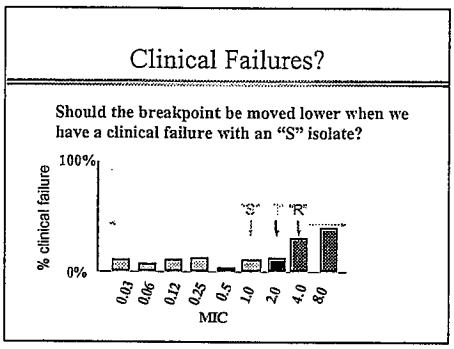
Should the breakpoint be moved lower when we have a clinical failure with an "S" isolate?



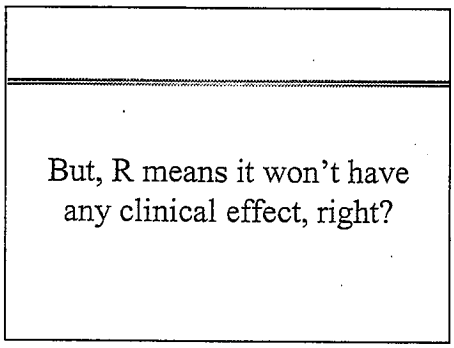
So let's take an example drug, and the S breakpoint is 1,intermediate is 2 and resistant is 4. So, if the organism is inhibited *in vitro* at 1 mcg/ml, you are going to get an S back. If not a 1 but a 2, you are going to get high, and if two doesn't get it, you are going to be a 4. You are going to be resistant, because there is going to be 4. So you have a clinical failure in a patient with an MIC of 0.25, and this has happened with *Salmonella* in fluorquinolones in humans, where some proposed that since they had a human patient that the treatment failure with an is olate like this, that the susceptible breakpoint for fluorquinolones in *Salmonella* in humans, you can move that to 0.25.



In reality, what you see is the steady baseline of failures along the susceptible range. You all know that there is much more to the clinical response of a patient than just whether or not the drug happens to be ..... And maybe I am letting students in on this too early, but the actual truth is, the thing that keeps us in some sort of standing in the community is many of the patients get better in spite of what we do. And so they are going to respond, but some of them are not going to respond regardless of what you do. For example, in respiratory disease in cattle, that is probably 5%, maybe even 10%. And if you set them right, at the intermediate, you start to see a little bump, and once you get above these, you start to see an increase in clinical failures. So that is the model we carry around on a good validated breakpoint.



So, the R means it won't have any clinical effect, right?



At the end of this when you go home, I do place on a non-validated breakpoint application, I've got a *Strep* out of the lung of a dog, and I have an ampicillin susceptibility result. There is absolutely no validation there whatsoever. If you look at that S and said, "Oh, I'll pull my favorite dose for ampicillin out of Plumb's and it will work." You have just completely unfound it, okay? Because that validation has not been done. I do put more weight on ruling potential drugs out because of an R than I do on having great clinical competence in those cases, because of this. So this is again, David McCleary, provided us with this, and I just find it fascinating, because it goes back to that computer record. This is from a series of clinical trials and these are just the ones treated with tilmicosin. In these comparative trials, there were 5 trials, 235 *M. hemolytica* isolates. These were nasopharyngeal isolates, so you can debate upon exactly how they matched the lungs, but these were pulled out of these trials, and they did susceptibilities, dilutional MIC's, remember that S is at 8. And 8, 16, and 32 are the breakpoints for tilmicosin. These were successes, so roughly 234 successes, they were simply pulled for signs of respiratory disease, and that definitive case definition were treated, and then at 72 hours they were blessed and sent back home and never seen again. That really shows that those breakpoints work, I mean they are all susceptible, and there is 1 that is greater than or equal to 32 that responded. If I ended the talk now, we would go home and be okay, and maybe I should as some people have suggested. These are ones that are relapses, and they were ones that were declared a clinical success at 72 hours, went home and again met the case definition. So you can see that it kind of meets... Okay, here are the failures; 2, 4, and 8. So let's put them all together. Success, relapse, failure. You know you could actually make an argument for the distribution; the failures are actually more to the left. It brings home the fact that these are big picture population type concepts for S, I and R. To take published pharmacokinetics, to take S, I or R, even for a validated breakpoint just to the validated breakpoint, I showed you the use of microbiological approach to it. To use these and say definitively it will be cured, or no, it won't work. For example, the ampicillin breakpoint is radically different from the *Strep* from the lung of the dog for *E. coli* in the bladder of that dog; because of the way those drugs eliminate them. I would look at an intermediate isolate..... End of tape

The ability to derive an "exact" dose from the PK/PD method is an illusion.

Pitfalls

- Published PK data vs. therapeutic monitoring
- Variation in susceptibility results
- Using single dose data instead of multiple dose adjusted data

Realistic use of Susceptibility Data