Multi-Drug Resistant Salmonella
Mindy Brashears

[1]Next let me introduce Dr. Mindy Brashears from Texas Tech University, International Center for Food Industry Excellence to discuss multi-drug resistant salmonella. Dr. Brashears is an Associate Professor and Director of the International Center for Food Industry Excellence in the Department of Animal and Food Sciences at Texas Tech University. Tremendous research experience and outstanding teaching have established her excellent recognition and reputation to our industry. Her current research emphasis is in the area of conducting research to address current food safety issues in both pre-harvest and post-harvest environments. Please help me in welcoming Dr. Brashears. Thank you Dr. Tiperetti. I want to begin by thanking the organizing committee for inviting me here today to speak on this topic. And also recognizing Dr. Guy Loneraganfrom West Texas A&M University and Dr. Karen Killinger Mann, who was one of my former students but recently left for a faculty position at Washington State University. And they assisted me with the presentation and proceedings. I am going to talk about multi-drug resistant salmonella. And what I really want to focus on is what we know and what we think we know. Because about three or four years ago, whenever we started doing research in multi-drug resistant salmonella, we thought we had a lot of theories and a lot of hypotheses and different ideas that were really good. And then we started collecting the data and we found out that what we thought would be going on whenever we fed animals antibiotics or when we looked at the prevalence, it's not necessarily what's actually going on. So in order to do that, we have to go back and look at some of, some of the basic patterns that happen whenever we look at the establishment of antimicrobial drug resistance. And we'll talk about that a little bit. And then I will share with you some data that we have collected over the last few years.

[2]Of course as you have heard over the course of the day that salmonella is the leading cause of death from food-borne illness. And it is one of the number one causes of food-borne illness in general. In 2002, salmonella Newport and salmonella Typhimurium were two of the top three human isolates. This is important because both of these are associated with multi-drug resistance. In this particular study, twenty-seven percent of Typhimurium were resistant to more than five antimicrobial drugs. And twenty-two percent of Newport were resistant to more than eight antimicrobial drugs.

[3]This, the topic of antimicrobial drug resistance has merged as the highest priority issue for public health officials in developed countries. As you have already heard, antimicrobial drug resistance increases the cost of healthcare also the morbidity of the illness. And as we know, resistance isn't new, but over the last few years we have seen an emergence of organisms that are of consequence as food-borne pathogens, specifically we have looked at salmonella and Campylobacter as having increased amounts of antimicrobial drug resistance and specifically multi-drug resistance.

[4]Just to give you a few examples, salmonella Typhimurium DT104 carries resistance on the chromosome. And it has the ACSSuT pattern which is resistance to five antibiotics. Salmonella Newport multi-drug resistant Amp-C also has the ACSSuT pattern as well as resistance to four to five other antibiotics and decreased susceptibility to several other antibiotics. Fluoroquinolone-resistant salmonella has been associated with poultry and fluoroquinolones are important because these are used to treat adults whenever they come down with a case of salmonella. And we have also seen Ceftriaxone-resistant salmonella which are important when treating children with cases of salmonella. So this is definitely an important aspect of our industry.

[5]How do we get antibiotic drug resistance? Well there are several mechanisms that are used by the bacteria to actually counteract the effect of the antibiotic. One is alteration of the drug target or bypassing the drug target by the DNA. When it's actually inactivation of the drugs, the bacteria can produce different compounds, chemicals, enzymes that deactivate the antibiotic. Reduced permeability

where the antibiotic cannot penetrate the cell membrane. They can increase the efflux, which means that whenever the antibiotic penetrates the cell membrane, they turn on pumps and actually pump out the antibiotic from the inside of the cell. And also this cell can increase the stress regulon response to the antibiotic.

[6]Now commonly we think that you feed an antibiotic or you expose the bacteria to an antibiotic and some type of mutation occurs. That cell mutates and then we have resistance. Which that does indeed happen, but actually this is probably not responsible for the majority of the cases. And we have found this out over the past several years of collecting data and actually looking at populations and quantifying the amount of, or quantifying the amount of total bacteria and then looking at the resistant bacteria.

[7]In most cases, what we have found is that the resistant populations are there in very low levels. We add some type of pressure. And this allows the low levels of resistant bacteria to actually grow and increase to the total population instead of seeing an actual mutation. In general we can classify resistance as intrinsic, which it's there naturally. Or it can be acquired by a number of means. And I'm going to briefly go over some of these different types of acquired resistance.

[8]First of all, acquired resistance can be done by genetic mutation, which is what I briefly mentioned earlier. And like I said, it does happen but it doesn't happen very often. One of the primary factors where we see a genetic mutation and an increase in resistance is fluoroquinolone resistance in salmonella. And this is a specific point mutation to the gyrA gene, which this gene actually causes super coiling in the bacteria and allows it to uncoil so we can get DNA replication. And we have a mutation to that. So the cell cannot replicate. Whenever we see this and we look at bacterial populations, you can see according to the graph you get a stepwise increase in the amount of resistance. It's not something that's dramatic. But over time, we'll just see more and more of the resistant bacteria in the population.

[9]Another means by which we can get resistance is acquisition of exogenous genetic material and we get a DNA exchange from the environment or from cell to cell. This occurs probably a lot more rapidly than the actual mutation in the cell. And it's done through mobile genetic elements. When this actually happens, we see a rapid increase in the number of resistant bacteria in our populations.

[10]This can be done through plasmids. And plasmids are a self-replicating extra chromosomal DNA. So they replicate faster than the bacterial DNA. And we can have multiple copies of a plasmid that carries the resistance in the bacterial cell. And this is represented by the yellow circle up there with the blue little piece in there which represents a resistance gene.

[11]And just to demonstrate, you can have e coli. This could be a generic e coli. It could actually be an Enterococcus. It could be anything naturally occurring in the animal in this or in the environment. And if it comes into contact with salmonella, it can actually be transferred through plasmid-mediated conjugation to a salmonella cell. So they're transferred across and within species.

[12]Also we have other genetic elements such as Transposons which are jumping genes which can jump out of the DNA whether it's in a plasmid or if it's on the chromosome of a bacterial cell. These can jump out, insert themselves in other bacteria or insert themselves in other places in the DNA of the cell. And this can also cause a significant amount of resistance.

[13]Integrons are also a way we get resistance from exogenous DNA. And this, these are actually allow us, bacteria to adapt to hostile environments. And when bacteria are exposed to an antibiotic or acid or some cold or heat stress, they are going to adapt. And that's why they have integrons. The integron is represented by the purple, the line on the bacterial DNA or plasmid DNA. And you have little pieces of DNA floating in the environment. The DNA, well actually the integrons come out. They take the DNA and incorporate it into the DNA of the, either the chromosome or

the plasmid.

[14]And this is just an example. You have this DNA. You have all these pieces which could actually be for various antibiotics,

[15]Various stress tolerant and they become incorporated into the bacterial cell.

[16]Some examples of when integrons actually are used or actually become important with regard to food safety and food microbiology, salmonella Typhimurium DT104 actually has two integrons. One is on SGI1, genomic island 1 and we have SGI2. And these actually are responsible for the resistance of our DT104.

[17]Another way that I'll mention that we can have resistance in an increasing resistance in our populations is through genetic propagation or horizontal transfer, which is also a form of genetic propagation in the environment. What happens in this case, whenever you purchase, whenever you go home from the doctor with an antibiotic, they always say take the entire antibiotic. And that's so you get rid of all of the bacteria in your system.

[18]Now this is what happens if you don't do that and you have some or if you have resistant. The red cell or the parent cell represents a resistant-organism. You take the antibiotic or an animal is exposed to an antibiotic. And this resistant population survives. It propagates and it becomes dominate, very simple concept.

[19]Now this resistant bacteria can also transfer the resistant genes through various means to another parent that might be susceptible. So the red could represent a population of e coli. The yellow could represent a population of salmonella. And you get transfer of resistance to the parent.

[20]And then you get propagation of two populations. So there's a lot of different means to acquire resistance and why we're seeing resistance in the food supply and in our environment.

[21]Some other things that come into play is that we need to realize if the animal or if a person or wherever in the environment is exposed to one antibiotic, we may select for other antibiotic resistance genes. For example, we could select for ampicillin and then we get multi-drug resistance. It's linked together with the genes for chloranfenicol, streptomycin, sulfamethoxazole, and tetracycline. So we have resistance to multiple antibiotics by exposing to only one.

[22]Also what we have found is you can expose bacteria in the lab to an antibiotic and then you also get resistance to other factors such as heat resistant. They're more heat resistant or they're more tolerant to acid. We've done a lot of work with lactic acid. So if you have a population of antibiotic resistant bacteria on a carcass. You treat it with the acid wash. Some of those may be more resistant to the acid as well because they're antibiotic resistant. So that comes into play as well from a food safety standpoint.

[23]Now there's a lot of perceptions about antimicrobial drug resistance. And these are just a few quotes that I'd pulled from various sources. And I'll read those. Salmonella serotype Newport multi-drug resistant AmpC was commonly identified in humans and food animals. These findings support the possibility of transmission of this organism to humans through the food chain. The next one is antibiotic-resistant strains of salmonella in the United States evolve primarily from livestock. Antimicrobial resistance in Salmonella and other food-borne bacteria is largely a consequence of the use of antimicrobial agents in food-producing animals.

[24]And one more, continued efforts to reduce unnecessary use of antimicrobial agents in food animals is critical to prevent further development of resistance to quinolones and cephalosporins. These are all quotes that have been thrown out there. But there is actually no evidence to show that using antibiotics in cows specifically is what we've looked at, has a direct link on what happens in the food

supply. These are broad sweeping statements, but we don't know if they're necessarily true. We have several studies underway to determine if feeding various antibiotics to animals actually results in an increase in antimicrobial drug resistance in the populations we isolate from animals.

[25]And just to give you one example and we've, this was touched on briefly in earlier in the, early mid-nineties I believe, DT104 prevalence was around thirty-five percent of isolates, thirty-four to thirty-five percent. Recently, it has dropped down to twenty-three percent without any really major changes in any production practices or anything to target, to cause that reduction. And there has actually been no associations directly made with DT104 on the farm and DT104 in animals. Now there was one publication in one, there was one publication that speculated that. But really the association was very weak. Another example is DT104 is disseminated very effectively in Norway and they have very stringent controls on the antimicrobial use but yet they still see an emergence of DT104, which has the multi-drug resistance genes. So making those associations are not always logical. We think in our minds, well if you add an antibiotic, you're going to get resistance. But that isn't always what happens.

[26]Now in cattle we do have different, antimicrobials are used in cattle. We have sub-therapeutic doses that are used to prevent liver abscesses and to improve feed efficiency. We have therapeutic doses that are administered at arrival to prevent infectious disease. And we also treat our animals when they get sick. The theory is that we could select for resistance that could be transferred to humans. But we have little understanding of the actual link of the use of antibiotics on the farm and how that transfers to humans.

[27]To give you an example of what's used on the farm, Monensin which is an ionophore is given to ninety-six point five percent of the cattle in feedlots today to improve feed efficiency. Tylosin is a macrolide which prevents protein synthesis. It's given to forty-two point three percent of cattle. And it is given to prevent liver abscesses. Some other ones are actually approved but they're not used as often.

[28]Some of our therapeutic drugs given to cattle are Tilmicosin, which is also a macrolide. It's given to prevent bovine respiratory disease as a metaphylaxis which means all the animals coming into the feedlot are given this injectible antibiotic in ten point four percent of our animals coming in. And fourteen point four percent of our animals are treated with this treatment for bovine respiratory disease. So almost all of our animals are exposed to some source of antibiotic.

[29]Now because of this, we have done some studies. We have one very large study going on right now. And I'm just, I don't have any slides on this but I'm going to give you just a brief overview of our results. We have a group of cattle that are fed sub-therapeutic doses of antibiotics. We have a group that are not. They're in a feedlot setting. This complete study is going on in a feedlot environment. We followed them throughout the entire feeding period, sampled them at arrival, at re-implant of around eight fifty-six. And then at slaughter we looked at the generic e coli, the Enterococcus in the salmonella. We found no difference in the antimicrobial drug susceptibility in any of our indicator organisms between the groups that were fed the low-dose antibiotics and the ones that were not fed. So we have, we did find resistance but the resistant populations were essentially the same in both groups, in all the gram positive, gram negative and in the salmonella. So we feel very good in those results, very confident. It was a very large study. It took about a year and a half to do several replications of that study. Another study we're working on and this is, this study is funded by NCBA and it's at Texas Tech, Washington State University and at the Meat Animal Research Center. And we're looking at just what we find as far as salmonella in beef plants and in cull, in cull animals we're looking at both cull dairy cows and cull beef cows. And in these studies we are looking at hide samples, taking samples from the hide and isolating the salmonella. And we're actually quantifying the amount of salmonella. We are also taking fecal pat samples from feedlots, looking at healthy harvest-ready cattle and then looking at pens of realizer animals, which these are chronically ill

animals where we have been sampling. We are going to sample on four seasons and we have data from two of the seasons that we can summarize.

[30]What we are looking for is both detection and quantification of the salmonella. What we have found is that yeah, we can always isolate salmonella. But how many are there? And so we quantify that. And then instead of isolating one single salmonella isolate per animal or per cow or for per fecal pat, we look at ten to fifteen different salmonella isolates. Because you have a lot of different serobars within one animal. It's a lot of work. It takes a lot of time. But we're getting a lot of valuable data from that. Then we send these off for serotyping by the NBSL. So we have a bank of salmonella for further characterization.

[31]To give you a brief overview of our data to date, in the fall of 2005 we had a sixty-three point three percent prevalence, number of animals positive. And again these are hide samples from fed beef. We had seventy-five point six and seventy-one point one in the cull, dairy, and beef animals. In the winter, our fed beef animals the prevalence was up at ninety-two point two percent. Our dairy and beef were at sixty-six point seven and at fifty percent. A little bit less in our cull animals as far as total prevalence. Once we determine the prevalence, we also quantified this, took the isolates. Sent them off for serotyping. Brought those back and did, used the NARMS panel for the sixteen panel antimicrobial drug resistance patterns.

[32]What we found, sorry these are from the feedlot, the fecal pats in our harvest-ready cattle. We had sixty-three percent in our cull animals, twenty-three percent. These are the realizers again that are chronically ill. In the winter, we had about twenty percent of our harvest-ready cattle in the fecal pats, in the feedlot and four point four percent of our cull animals were salmonella positive. Like I said in the beginning, I wanted to talk about what we know and what we think we know. Our salmonella consistently, all across the board from all of our studies has been less as far as total prevalence and our quantification data, you'll see in a minute, in the amount of salmonella.

[33]Another important topic or another important observation is the fact that we did not see a higher prevalence of multi-drug resistance in our cull animals and our beef animals. You can see now we classified and we had a discussion at the Food Safety Summit at NCB8, how do you classify multi-drug resistance. We called it multi-drug resistance if it had two or more, if it was resistant to two or more antibiotics. You will see that most of these are not resistant. In our fed beef, we had three point three percent, cull dairy two point two and in our cull beef, three point three percent. This is in the fall. The number of drugs they were resistant to and the ones that were resistant average of two point three. So just over two for our beef and an average of one drug more for our cull animals. So a little more resistance in those animals. In the winter samples we did have a higher prevalence or a higher number of antibiotic resistance in our cull beef animals, thirty-six point six percent. And about the same, the fed beef in the cull dairy. Again, not a huge difference in the number of drugs they were resistant to. So even though we are finding resistance, we're not seeing a great increase in the number of drugs they're resistant to. They're still only resistant to two or three.

[35]This is our quantification data. You can see our fed beef had about a log of one point five. This of course was done using an MPN method. The cull dairy and the cull beef were both at a log of less than one. In our fecal samples, we, which you can't do a direct comparison of these cause the hide samples were per forty square centimeters. And the fecal samples are log CFU per gram. So don't do a direct comparison of the two. But we had almost a log of four in our healthy feedlot and about a log of one point five in our realizer animals. So the total salmonella present was much lower.

[36]Another important finding of this study is, and this was similar to some of the swine data we saw earlier is if you look at the common causes of human illness, which are Typhimurium, Enteritidis, Newport and you can read down the list on the right-side of the page and compare that to the serobars that we're actually finding, they're not the same. We do not find serobars of importance in human medicine in

our cattle. What every single isolate has gone for serotyping, we have not found a single Typhimurium or a single Newport. And there's very little overlap with the list on the left and the list on the right.

[37]So preliminary findings show that we do have salmonella in the Texas High-Plains. We do find it commonly. We were not surprised at the prevalence of salmonella by any means. We always find that on hides. It's frequently recovered from our healthy cattle. Multi-drug resistance, especially if you look at high level multi-drug resistance, five or more, eight or more is very rare in all of our groups, whether they be health animals or the cull animals. Sixty-eight percent of our isolates were susceptible to all the antibiotics we tested. And only one point seven percent were resistant to four or more antibiotics. Some of our higher level, multi-drug resistance is most common in our cull animals, the four or more. But we still didn't see a huge difference in our cull animal populations of resistance in our healthy animals. And our serotypes that we recovered are unlikely associated with human disease.

[38]The bottom line, take-home message is that acquisition emergence and maintenance of antimicrobial drug resistance is very complex. There's a lot of things going on. When we take a research approach to this, we're having to quantify it. We need to find really good methods to quantify our resistant populations compared to our non-resistant populations. We're working on that. You cannot isolate one salmonella per animal per fecal pat per sample. We are finding multiple serobars of salmonella per animal. Some of them are resistant. Some of those aren't. We're getting more and more data on that. I didn't present that cause we're just starting to get that from our summer isolates. We do know that from our studies that resistant in especially high level multi-drug resistance in animals is uncommon. And antibiotic usage may select for resistance but there are other factors as well that can select for resistance. It's not just antibiotic use because in our animals that are not fed any low level antibiotics, have not been exposed. In that study, we pulled out any animal that had to be treated. So those were all pulled out of our population. So with no antibiotic exposure, we're still seeing the resistance pattern. So there's something else that's causing the emergence of our salmonella and even our gram positive and gram negative indicators in those animals. So it is very complex. How it happens, what's going on and it can be controversial. There are many unknowns. We're trying to find out some of these answers through our research. And hopefully over the next two to three years we have five studies going on right now addressing these issues. And hopefully as we get more data, we're going to have more of the answers and be able to answer more of the questions.