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Antibiotics, Bacteria, and Antibiotic Resistance Genes: Aerial Transport from Cattle Feed Yards via Particulate Matter

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Short title**: Antibiotic resistance from cattle feedyards**

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Abstract

Background: Emergence and spread of antibiotic resistance has become a global health threat and is often linked with overuse and misuse of clinical and veterinary chemotherapeutic agents. Modern industrial-scale animal feeding operations rely extensively on veterinary pharmaceuticals, including antibiotics, to augment animal growth. Following excretion, antibiotics are transported through the environment via runoff, leaching, and land application of manure; however, airborne transport from feedyards has not been characterized.

Objectives: The goal of this study was to determine the extent to which antibiotics, antibiotic resistance genes (ARG), and ruminant-associated microbes are aerially dispersed via particulate matter (PM) derived from large scale beef cattle feedyards.

Methods: PM was collected downwind and upwind of ten beef cattle feedyards. Upon extraction from PM, six veterinary antibiotics were quantified via LC-MS/MS, ARG were quantified via targeted qPCR, and microbial community diversity was analyzed via 16S rRNA amplification and sequencing.

Results: Airborne PM derived from feedyards facilitated dispersal of several veterinary antibiotics, and microbial communities containing ARG. Concentrations of several antibiotics in airborne PM immediately downwind of feedyards ranged from 0.5-4.6 μg/g of PM. Microbial communities of PM collected downwind of feedyards were enriched with ruminant-associated taxa, and were distinct when compared to upwind PM assemblages. Furthermore, abundance of genes encoding resistance to tetracycline antibiotics was significantly greater in PM collected downwind of feedyards as compared to upwind.

Conclusions: Wind dispersed PM from feedyards harbors antibiotics, bacteria, and ARGs.

Introduction

Bacterial resistance to antibiotics increasingly hinders treatment of life-threatening illnesses. Misuse and overuse of antibiotics plays a critical role in development of resistance and there is evidence that agricultural use of antibiotics is a contributor to the aggregation of resistance in the environment (Gilchrist et al. 2007; Levy and Marshall 2004). Nearly 10 million kg of antibiotics per year (likely an underestimation due to lack of reporting requirements) are used in animal agriculture in the United States alone (Mellon et al. 2001; Sarmah et al. 2006). Antibiotics are administered to beef cattle to treat and prevent disease and to promote growth (Khan et al. 2008; Phillips et al. 2004; Shuford and Patel 2005). Antibiotics used for growth promotion are added to livestock feed, and upon ingestion are incompletely metabolized and poorly absorbed in the gastrointestinal tract resulting in excretion of parent compounds and metabolites (Boxall et al. 2006; Chee-Sanford et al. 2009; Khan et al. 2008; Shuford and Patel 2005; Wegener 2003). Upon excretion, these compounds may be transported into the environment beyond feedyard boundaries via application of manure waste onto agricultural fields, runoff, and, as reported here, via airborne particulate matter (PM) (Chee-Sanford et al. 2009; Wegener 2003). Once in the environment, antibiotics can facilitate *de novo* development of bacterial antibiotic resistance and provide a selective advantage for bacteria that acquire resistance either in treated animals or in the environment (Chee-Sanford et al. 2009; Gilchrist et al. 2007; Silbergeld et al. 2008).

In 2012, there were 2,100 large-scale (greater than 1,000 head of cattle) beef cattle feeding operations in the United States (NASS 2013). As of March 1, 2014, 76.3% of all cattle residing on US feedyards with more than 1,000 head (8.24M cattle) were located in Texas, Oklahoma, Kansas, Nebraska, and Colorado (NASS 2014). Together, portions of these states constitute a region which has the highest frequency of dust storms in the United States (Orgill and Sehmel

1976) and the highest density of feedyards. Climatic conditions in this semi-arid region are conducive to wind scouring of dry soils, and aerial transport and deposition of PM onto surrounding soil surfaces, water surfaces, vegetation and other living organisms. Moreover, feedlot cattle behavior facilitates daily suspension of PM above feedyards (see Supplemental Material, Figure S1). Relative humidity and soil moisture levels in this region are typically highest in the early morning hours and decline throughout the day. As a consequence, feedyard pen floor material, which consists primarily of urine and fecal material, becomes dry and brittle, thus becoming source material for fugitive dust (Von Essen and Auvermann 2005). Despite management practices employed at many feedyards such as landscaped windbreaks, frequent pen scraping, and sprinkling to mitigate dust production, cattle activity and movement in late afternoon and early evening results in pulverization and subsequent aerosolization of pen floor material. Wind speeds and temperatures are generally lowest early in the day, increasing throughout the afternoon, and decreasing again in the evening hours. Stable atmospheric conditions with minimal vertical mixing and turbulence also facilitate suspension of PM into air above feedlots. Fronts and other major weather patterns frequently sweep through this region, and are often associated with exceedingly high wind velocities which themselves transport significant masses of particulates into the atmosphere and across the region and continent (see Supplemental Material, Figure S2). Thus, in semi-arid regions where a majority of beef cattle feedyards exist in North America, transport of livestock-generated organic wastes occurs largely via wind. Under certain conditions, the Central Plains region of the United States becomes a large open source area producing dust on a scale commensurate with its vast size (Stout 2001).

People living in the vicinity of feedyards often complain about excessive dust, and airborne microorganisms and by-products from feedyards are considered potential human health threats

(Dungan 2010). Yet the health of feedyard workers and nearby residents has not been thoroughly assessed (Von Essen and Auvermann 2005). There is increasing potential for human exposure to airborne dusts and associated antibiotics and microbes as population centers grow (Dungan 2010). Further, there appears to be consensus among numerous climate models that the American Southwest will continue to dry throughout the 21st century, potentially returning to Dust Bowl era and 1950's drought conditions (Seager et al. 2007). Although extended periods of drought may result in reduced density of feedyards in affected regions, trends within the industry suggest that future consolidation of small feedyard operations into larger, corporate-owned operations will increase, particularly in arid regions where conditions are ideal for beef production (Galyean et al. 2011). If so, exposure to feedyard-derived dusts, antibiotics, and microbes will increase, not only via direct inhalation, but also from deposition onto skin, food, and into water (Dungan 2010).

Given the extent of confined beef cattle production, extensive use of veterinary antibiotics, and significant wind energy potentials in the Central Plains Region of the United States, we hypothesized that antibiotics and antibiotic resistant bacteria in PM collected downwind of beef cattle feedyards would be abundant compared to that in corresponding upwind PM. To address our hypothesis, we quantified antibiotics commonly administered in animal-based agriculture, assessed microbial community composition, and assessed occurrence of antibiotic resistance genes in airborne PM emanating from beef cattle feeding operations.

Methods

Sample collection

Particulate matter samples were collected adjacent to ten commercial beef cattle feedyards in the Southern High Plains. Feedyards included in this study had capacity of 20,000-50,000 head of cattle and were selected based on size and accessibility, including being within a 200 mile radius of Lubbock, TX. Two feedyards (feedyards 7 and 8) sampled in this study were within one mile of each other but were not downwind of one another at the time of sampling. The remaining eight feedyards in this study were not within 5 miles of another feedyard. PM was collected using Hi-Q CF-902 Digital portable high volume air samplers to collect total suspended particulates (TSP) on four inch glass fiber filters (HI-Q Environmental Products) for 30 minutes upwind and downwind of feedyards, approximately 10-20 m from feedyard boundaries at a height of 2-3 m above the ground to obtain sufficient mass for analytical detection. Filter cartridges and holding containers were washed with 70% ethanol after each use. Sampling occurred in the late afternoon between 16:00-20:00 when cattle are most active and the greatest amount of PM is produced (Purdy et al. 2007). Sampling occurred between August and December 2012, and during weather conditions that were similar at all feedyards and characterized by moderate temperatures (mean= 18±2 °C), slight winds (mean= 3.0±0.5 m/s), low relative humidity, and no precipitation (see Supplemental Material, Table S4). All PM samples were transported to the laboratory on ice, weighed upon arrival to determine PM mass, and then frozen at -80 C until analysis.

Quantitation of veterinary antibiotics in TSP

Extraction and clean-up

Veterinary antibiotics have been previously identified and quantified using high performance liquid chromatography with tandem mass spectrometry (LC-MS/MS) (Aust et al. 2008; Kim and Carlson 2007). Particulate matter samples were analyzed following the method of Kim and Carlson (2007) with minor adaptations. Briefly, entire filters were placed in 40 ml polypropylene tubes and twice extracted with 40 ml of ammonium hydroxide buffer solution (pH 10) or McIlvaine buffer solution (ph 4), depending on target compound, and 200 µl K_2EDTA. Samples were filtered through syringes with DIONEX ASE cellulose filters before being loaded on to pre-conditioned Strata-X High Performance Polymeric SPE cartridges for cleanup (Phenomenex). Cartridges were eluted with methanol, evaporated to dryness, and reconstituted in 1 mL of 10:90 methanol:water plus 0.1% formic acid.

LC-MS/MS parameters

Liquid chromatography with tandem mass spectrometry (LC-MS/MS) using electrospray ionization (ESI) has been utilized for the identification and quantification of antibiotics in a variety of matrices (Hirsch et al. 1998; Kim and Carlson 2006; Kim and Carlson 2007; Luo and Ang 2000). Target compounds were separated via reserve-phase chromatography utilizing a 100X2.1 mm Kinetex PFP column with a 1.7 µm particle size and a Thermo Accela 1250 solvent pump equipped with a PAL autosampler. Mobile phase conditions were identical for all target compounds in the analysis (see Supplemental Material, Table S1). Tandem mass spectrometry utilizing ESI on a Thermo TSQ Quantum Access Max system in positive ion mode was used for identification and quantification of target compounds. Tandem mass spectrometry operating conditions were optimized for each compound and the two most prevalent product ions were

used for target compound identification and quantification (see Supplemental Material, Table S2).

DNA extraction

DNA was extracted from PM bound to filters using a PowerSoil DNA Isolation Kit with minor adaptations (MoBio Laboratories). In lieu of soil, slices of each filter containing bound PM were taken from the center of the filters and added to the PowerSoil bead tube. Additionally, PM that had become dislodged from filters while in storage was included in the PowerSoil bead tube for extraction. Extraction proceeded per the directions in the kit resulting in 100 µl of DNA in elution buffer (10 mM Tris). Concentrations of DNA in each sample were measured and recorded using a NanoDrop Spectrophotometer (Thermo) to account for total DNA used in qPCR.

Real time PCR assays for detection of resistant genes

Quantitative polymerase chain reaction (qPCR) was used to detect the presence of nine antibiotic resistant genes. Previously published primer sets for six tetracycline resistant genes (Peak et al. 2007; Smith et al. 2004) were used for qPCR assays (see Supplemental Material, Table S3). Of the six tetracycline resistance genes targeted in this study, four (TetM, TetO, TetQ, and TetW) encode ribosomal protection proteins that remove tetracycline from the ribosome in a GTP-hydrolysis-dependent fashion, and two (TetB and TetL) code for cellular efflux proteins as their antibiotic resistant mechanisms (Peak et al. 2007). Selected resistant genes were included in this study based on their previously reported detection in waste lagoons proximate to feedyard operations (Peak et al. 2007; Pei et al. 2006; Smith et al. 2004; Wu et al. 2010) and because of heavy veterinary use of tetracycline and sulfonamide antibiotics (Hamscher et al. 2002; Kemper

2008; Lindsey et al. 2001). qPCR analyses were performed on Roche Lightcycler 480 instruments using LightCycler 480 DNA SYBR Green 1 Master Mix consisting of FastStart Taq DNA Polymerase, dNTP mix, and SYBR Green I dye. Temperature cycling was modeled after that of Pei, et al (Pei et al. 2006), and entails one cycle of denaturation at 95 C for 10 minutes, followed by amplification at 35 cycles of 15s at 95 C and 1 min at 60 C for all nine gene-specific reactions. Due to the potential for erroneous amplicon production within the last five cycles of the reaction, target genes were considered present in the sample when amplified before 30 cycles of qPCR. Amplicons produced through qPCR were each verified to be single amplicons by agarose gel electrophoresis followed by Sanger sequencing to verify amplification of the correct, unique DNA sequence. Melt curve analysis also verified single amplicon generation in each qPCR reaction. Amplicons in samples were quantified using a standard curve derived from amplification of known template amounts of synthesized oligonucleotide for each unique amplicon (Integrated DNA Technologies).

Microbial diversity analysis

The relationship between microbial communities present in PM upwind and downwind of feedyards was determined through DNA pyrosequencing. Samples were analyzed following 16S rRNA amplification and sequencing as described by Dowd et al (2008), targeting the V1–V3 regions of the 16S rRNA gene Analysis was performed at Research and Testing Laboratory, LLC, Lubbock, Texas (http://www.researchandtesting.com).

Bioinformatics

Any sequence that contained a low quality barcode or that failed to be at least half the expected amplicon length (or 250 bp, whichever was shortest) was removed from the data pool. As such,

two samples were removed from analysis because of an insufficient number of quality sequencing reads. All sequences then were denoised using an algorithm based on USEARCH (Edgar 2010) and checked for chimeras using UCHIME (Edgar et al. 2011). The sequence data processing pipeline followed that outlined by Research and Testing Laboratory. After denoising and chimera checking, sequence data were separated into operational taxonomic units (OTUs) and annotated using the RDP classifier (Wang et al. 2007) with GreenGenes v. 12.10 (McDonald et al. 2012) used as a reference. Finally, relative abundances of taxa at each hierarchical taxonomic level were calculated using the summarize_taxa.py QIIME script.

Data analysis

Microbial communities present in PM were analyzed to determine if differences existed between downwind and upwind PM. The number of OTUs was used as a measure of bacterial species richness, and was standardized to a consistent sampling effort using rarefaction prior to analysis. Differences in richness between upwind and downwind samples were evaluated using a paired t-test. Weighted UniFrac distances (Lozupone et al. 2006), which measure phylogenetic distances among samples, were used to characterize beta diversity among all samples, and were illustrated using Principal Coordinates Analysis (PCoA). Group differences (upwind vs. downwind) with respect to the overall microbiome were evaluated statistically using distance based redundancy analysis (Anderson and Willis 2003). Here, within and among group patterns of beta diversity were examined using an ANOVA-like permutation test available in the 'vegan' R package (Vegan: Community ecology package, R package version 1.17-1). To further categorize differences between upwind and downwind PM samples, paired t-tests were used to determine the statistical significance of differences in total PM mass, differences in the most abundant genera and phyla observed, as well as differences in antibiotic concentrations. UniFrac distances

and rarefaction analysis were calculated using QIIME (http://www.qiime.org) and all analyses were performed in R (R Development Core Team Version 2.14.1).

Results

Particulate matter and antibiotic concentrations

Mass of PM collected immediately downwind of feedyards was significantly different than that collected immediately upwind of each feedyard ($p=0.002$) (see Supplemental Material, Table S5). Utilizing both a high and low pH extraction buffer, extraction efficiency was maximized to the extent possible given the complex matrix of feedyard-derived PM. Monensin, a polyether ionophore antibiotic, was detected in 100% of PM samples downwind and upwind of feedyards, albeit below limits of quantitation in PM samples collected upwind of feedyards. Downwind of feedyards, mean monensin concentration was 1800 ± 370 ng/g PM (mean ± SE). Tylosin, a macrolide antibiotic, was detected in 80% of PM samples downwind of feedyards and the mean concentration was 340 ± 92 ng/g PM, significantly lower than concentrations of monensin across all feedyards.

Three tetracycline antibiotics (tetracycline, chlortetracycline, and oxytetracycline) were detected together in the majority of PM samples downwind of feedyards (60%) while oxytetracycline was the most frequently detected of the three and was detected in 100% of PM samples collected downwind of feedyards. Mean concentrations were 280 ± 170 ng/g PM tetracycline, 820 ± 220 ng/g oxytetracycline, and 970 ± 430 ng/g chlortetracycline. Additionally, oxytetracycline was detected in 30% of upwind samples at concentrations below the limit of quantitation. Overall, monensin was present at the highest concentrations, followed by chlortetracycline and

oxytetracycline, in downwind PM (Figure 1). One particular site, Feedyard 4, had consistently elevated concentrations of all antibiotics compared to other feedyards.

Bacterial community structure analysis

A total of 8,986 16S Operational Taxonomic Units (OTUs), based upon amplicon sequencing of the V1-V3 variable regions of ribosomal RNA genes, were observed across all samples. The number of PM-associated OTUs was significantly higher among downwind samples as compared to samples collected upwind of feedyards ($p=0.0095$), indicating greater diversity in PM downwind of feedyards (see Supplemental Material, Figure S4). Additionally, bacterial phyla and genera common to fecal matter and gut flora were significantly more abundant in PM downwind of feedyards compared to PM collected upwind (see Supplemental Material, Figures S5 and S6). Within these PM-associated bacterial communities were several genera that contain sub-taxa known to be infectious in humans such as *Corynebacterium* (present in 90% of all samples including 100% of downwind and 80% of upwind samples), *Leptospira*, *Clostridia*, *Bacteroides*, and *Staphylococcus*.

Multivariate assessment of microbial communities

UniFrac distance measures, which incorporate phylogenetic divergence into distances between samples, and Principal Coordinate Analysis (PCoA) were used to assess overall microbial community composition between PM samples collected upwind and downwind of feedyards (Figure 2). Microbial communities in PM samples collected downwind of feedyards tended to be more similar to one another than upwind samples, as depicted by 95% confidence intervals represented by ellipses. Bacterial community composition differed significantly between downwind and upwind samples ($p=0.005$).

Abundance of antibiotic resistance genes

Abundances of six targeted tetracycline resistance genes were significantly (all $p<0.002$) more abundant in PM collected downwind of feedyards compared to upwind (Figure 3). Additionally, tet Q and tet W were most prevalent across all feedyards (see Supplemental Material, Figure S7). No significant correlation between tetracycline concentrations in PM and tetracycline resistance gene abundance was noted.

Discussion

To our knowledge, this study is among the first to detect and quantify antibiotics and antibiotic resistance genes, and characterize microbial communities associated with airborne PM emitted from beef cattle feedyards. Yet, TSP samples collected at feedyard boundaries may not adequately represent particulates blown downwind from the source or those occurring at higher altitudes (Bonifacio et al. 2012; Hiranuma et al. 2011; McGinn et al. 2010). Coarse particles, and those with Aerodynamic Equivalent Diameters (AEDs) greater than 10 μm tend to settle more quickly than smaller particles. Thus, larger particles may impact areas adjacent to feedyards to a greater extent than smaller, fine particles. Hiranuma and colleagues (Hiranuma et al. 2011) examined dissipation of PM downwind of a feedyard in the Texas Panhandle to a distance of 3.5 km. PM_{10} concentrations were reduced to approximately 8.5% of concentrations recorded at the feedyard boundary. However, fine mode particle concentrations declined less than 1% after 3.5 km, leading the authors to note that even though fine mode particles contributed less to total PM mass, they would be expected to disseminate over a much broader area. Purdy et al (2007) also attributed lingering suspensions of feedyard dust in air to small geometric mean sizes of particles occurring within a range of 0.655 to 0.714 μm.

Feedyards generate significant masses of PM daily. There have been numerous attempts to estimate daily PM_{10} emission factors for beef cattle feedyards, and the range (4.6-127 g/animal/day) of estimates clearly illustrates the variability in both estimation techniques and in the measure itself (McGinn et al. 2010; Parnell et al. 1994). Recently, Bonifacio et al (2012) estimated emission factors for two feedyards in Kansas to be 27 and 30 g/animal/day. Assuming an average emission rate of 28.5 g PM_{10}/animal/day, then 234,840 kg of PM_{10} would have arisen from the 8.24M cattle on feedyards located in Texas, Oklahoma, Kansas, Nebraska, and Colorado each day during March 2014 (NASS 2014). Sweeten et al (1998) suggested that $PM_{2.5}$ (particulates with AED $\leq$ 2.5 μm that can be inhaled deeply into lungs) only represented about 5% of TSP. Data from a separate study in our laboratory which examined anabolic steroids associated with PM from feedyards indicated that 9.17% of PM_{10} collected from feedyards had an AED of $\leq$ 2.5 μm. Given this information, we can predict that the mass of $PM_{2.5}$ arising from feedyards in these five states alone would exceed 21,000 kg/day. Assuming fine mode particulates from feedyards contain similar concentrations of antibiotics, microbes, and antibiotic resistance genes as quantified in TSP samples collected at feedyard boundaries, and that antibiotics and microbes associated with PM are not destroyed during atmospheric transport, this mass of $PM_{2.5}$ released daily into the air may have significant, far-reaching implications for spread of antibiotic resistance.

Since the antibiotics quantified in this study have potential to be broadly distributed in the environment, it is important to consider their environmental fate and half-lifes. Reported half-lifes of tetracycline antibiotics in soil and soil-slurry mixes range from 30-180 days, while half-lifes in water are 15-30 days (Kühne et al. 2000). Monensin can remain in manure for greater than 70 days (Donoho 1984), whereas tylosin degrades within 30 days (Loke et al. 2000).

However, tetracycline compounds and tylosin bind more tightly to soil particles than monensin and likely also remain tightly bound to airborne particulates (Sarmah et al. 2006). Given the half-lifes associated with antibiotics identified in this study, it is feasible that they remain active during aerial transport and after deposition onto soil, water, or other surfaces for days to weeks.

Concentrations of antibiotics in airborne PM in this study were within ranges of antibiotic concentrations reported in PM collected inside large scale swine production houses (Hamscher et al. 2003). Swine production facilities are typically fully enclosed, which confines PM and leads to much higher indoor air PM concentrations. Beef cattle feedyards are open air facilities, which facilitate environmental dispersal of PM via wind. Additionally, antibiotic concentrations observed in this study were similar to those reported in cattle manure and soils adjacent to beef cattle production facilities (Aust et al. 2008; Christian et al. 2003) as well as those in swine manure (Zhu et al. 2013), suggesting a correlation between antibiotic use and resulting airborne PM-associated veterinary antibiotics. Detections of antibiotics upwind of feedyards were below limits of quantitation, but antibiotic presence upwind indicates that perhaps non-uniform dispersal of PM occurs. In many instances, feedyards used for this study were located in the vicinity of other feedyards. Thus, we cannot discount the possibility of collection of antibiotic residues, microbes, and resistance genes traveling aloft from distant upwind feedyards or from the same feedyard originating from past events and subsequent shifts in wind direction. While our sampling efforts were designed to maximize dust collection while minimizing sampling time, it should also be noted that the conditions under which we sampled were rather typical of those which occurred each day at feedyards. Therefore our samples are representative of those which would occur within the season and time periods at feedyards across our study area, but likely

different (i.e. lower dust, bacterial, antibiotic, and ABR gene sequence concentration) than those occurring at other times and during unstable weather.

Bacterial community composition and relative abundance of phyla were similar to that previously reported for cattle fecal matter (Ouwerkerk and Klieve 2001; Rice et al. 2012). In particular, Firmicutes and Bacteroidetes phyla dominated downwind PM samples as expected since they are the two most abundant phyla in cattle fecal matter (Rice et al. 2012). Furthermore, microbial community composition of PM collected immediately downwind of feedyards was distinct from PM collected immediately upwind, confirming the input of feeding operations to bacterial assemblages in downwind environments (Figure 2). Species richness of PM samples evaluated in this study was similar to individual ambient urban air samples (Franzetti, et al. 2010), however it is noteworthy that the OTUs observed in upwind PM tended to be highly variable across the ten feedyards while OTUs observed downwind of feedyards were substantially less so. This illustrates the degree of bacterial input from feedyards themselves to downwind PM as well as the variability of activities and sources upwind of feedyards contributing to the PM bacterial composition.

This study clearly demonstrates the potential for antibiotics and bacteria to be transported from beef cattle feedyards into the environment by wind. Thus, it is reasonable to consider how far microbes may be transported from these sources, and if they remain viable after aerial transport. This study was not designed to address these questions directly, rather it was intended to quantify airborne antibiotics, changes in microbial community composition, and identify antibiotic resistance genes derived from a potential source. Nonetheless, other researchers have documented the potential for dust and associated microorganisms to span great distances. For example, transatlantic movement of dust from Africa to Florida, a distance of over 6,500 km, has

been reported. Interestingly, 99% of those foreign particulates were between 0.3 and 1.0 μm (Griffin 2007). Air samples collected from multiple aircraft over San Antonio, TX in 1965 revealed a stable microbiological population at high altitudes (3,127 meters), and revealed that microbial abundance was heavily influenced by temperature inversions and frontal activity (surface and atmospheric turbulence) (Fulton 1966a, b). It has been estimated that more than 25% of total airborne particulates occurring in the atmosphere above land masses may consist of microorganisms and organic matter (Jones and Harrison 2004).

Atmospherically suspended microbes are susceptible to a variety of meteorological factors including UV-light, temperature extremes, and limited moisture. Cole et al (2008) suggested that the reason gram-negative pathogenic bacteria could not be cultured from open-air feedyard samples (as reported in Purdy et al. 2007 and Wilson et al. 2002) was because they were quickly killed by irradiation and desiccation. Yet these studies reported successful culture of numerous other microbial genera from airborne particulates. Griffin (2007) hypothesized that upper altitude dust may attenuate the antimicrobial effect of solar irradiance among bioaerosols suspended at lower altitudes during dust transport. At least one incident of transcontinental transmission of meningococcal meningitis via a dust cloud has been reported (Griffin et al. 2001). Griffin (2007) also noted that long range movement and survival of microbes (some of which are pathogenic) in the atmosphere should be expected based on their ubiquity and adaptability, and that dust likely plays a significant role in biogeographical distribution of pathogenic and nonpathogenic species. However, it is likely that degradation kinetics of antibiotics and ABR gene sequences and survivability of bacteria would be different under conditions with greater solar radiation and temperature.

Genes encoding resistance to tetracycline and sulfonamide antibiotics have been detected in soils adjacent to swine production facilities (Wu et al. 2010), in wastewater lagoons near beef cattle feeding operations (Peak et al. 2007), and in surface waters within a watershed dominated by agricultural facilities (Pei et al. 2006). To compare resistance gene concentrations to other studies, gene concentrations were normalized to units of gene copies per 16S copies. When normalized, tetracycline resistance gene concentrations in lagoon water (Peak et al. 2007) and surface waters (Pei et al 2006) were lower by several orders of magnitude than concentrations quantified in PM in this study, indicating that genes encoding resistance can indeed be more prevalent in PM (on a normalized scale) than other environmental matrices. Interestingly, resistance genes were detected in PM collected upwind of feedyards, albeit significantly less abundant. This indicates the potential for dispersal of feedyard-associated PM counter to prevailing winds on less windy days, or from distant upwind feedyards.

PM-associated antibiotic resistance genes from beef cattle feedyards are of potential concern due to the possibility of lateral gene transfer among the bacterial community (Chee-Sanford et al. 2009). Once in the environment, resistance genes may be transferred between bacteria across a variety of environmental matrices, from soil to PM or PM to surface waters, for example. While many of the veterinary antibiotics approved for use in beef cattle production are not intended for human use, the potential for resistance to multiple antibiotics within the same class or otherwise, including those intended for human use, has been documented (Chee-Sanford et al. 2009). Resistance genes, once in the environment, could be transferred to both non-pathogenic and pathogenic bacteria. This is especially important to consider when taking in to account the significant number of wind events that occur across the central United States and specifically in

the Southern High Plains, where this research was conducted. Large wind events are capable of global-scale transport and dissemination of PM and microbes.

Conclusion

In conclusion, PM generated at beef cattle feedyards contains distinct communities of bacteria, antibiotics, and antibiotic resistance gene sequences. Thus there is significant potential for widespread distribution of antibiotics, bacteria, and genetic material that encodes antibiotic resistance via airborne PM as a result of the large mass of fine particles released daily from beef cattle feedyards in the Central Plains of the United States. Dispersal of PM is facilitated by significant wind energy potentials and frequent wind events in this region. It follows that feedlot derived microbes, including those possessing antibiotic resistance, can be transported to new locations where they may occupy new niches (Griffin 2007).

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Figure Legends

Figure 1. Concentrations (in ng antibiotic per gram of PM) of five targeted veterinary antibiotics in PM collected immediately downwind of feedyards (n=10). Individual points are concentrations from each feedyard. Boxes extend from the 25th to the 75th percentile, solid horizontal lines represent the median of each distribution, and dashed horizontal lines represent the mean. Feedyard 4 (indicated by the number 4) had consistently elevated concentrations of antibiotics. TYL=Tylosin, MON=Monensin, TC=Tetracycline, OTC=Oxytetracycline, CTC=Chlortetracycline.

Figure 2. Biplot illustrating the Principle Coordinates Analysis (PCoA) of microbial assemblages in airborne PM samples collected upwind and downwind of feedyards located in the Southern High Plains, USA. Here, each point represents a sample, and samples from the same feedyard are linked together via a light blue line. Two upwind samples were removed from analysis because of an insufficient number of quality sequencing reads. The proximity of points in this biplot is an approximation of similarity of samples with respect to their microbiome composition. Similarities were measured using the weighted UniFrac measure, which quantifies the phylogenetic distances among samples. Ellipses represent the 95% confidence ellipse around group (upwind vs. downwind) centroids (i.e., multivariate means), and the arrows represent the correlation of individual phyla with PCoA axes. The percent of variability in UniFrac distances accounted for by each axis is denoted in the axis titles.

Figure 3. Mean fold increase (resistance gene copies downwind/upwind) of tetracycline resistance gene abundance in PM collected immediately downwind and upwind of feedyards (n=10). Fold increases demonstrate that six targeted tetracycline resistance genes were significantly more abundant in PM downwind of feedyards than upwind (denoted by *, all $p<0.002$), ranging from 100 fold to 1000 fold increase in abundance. Resistance gene Tet M had the highest fold increase in abundance between PM collected downwind and upwind.

Figure 1.

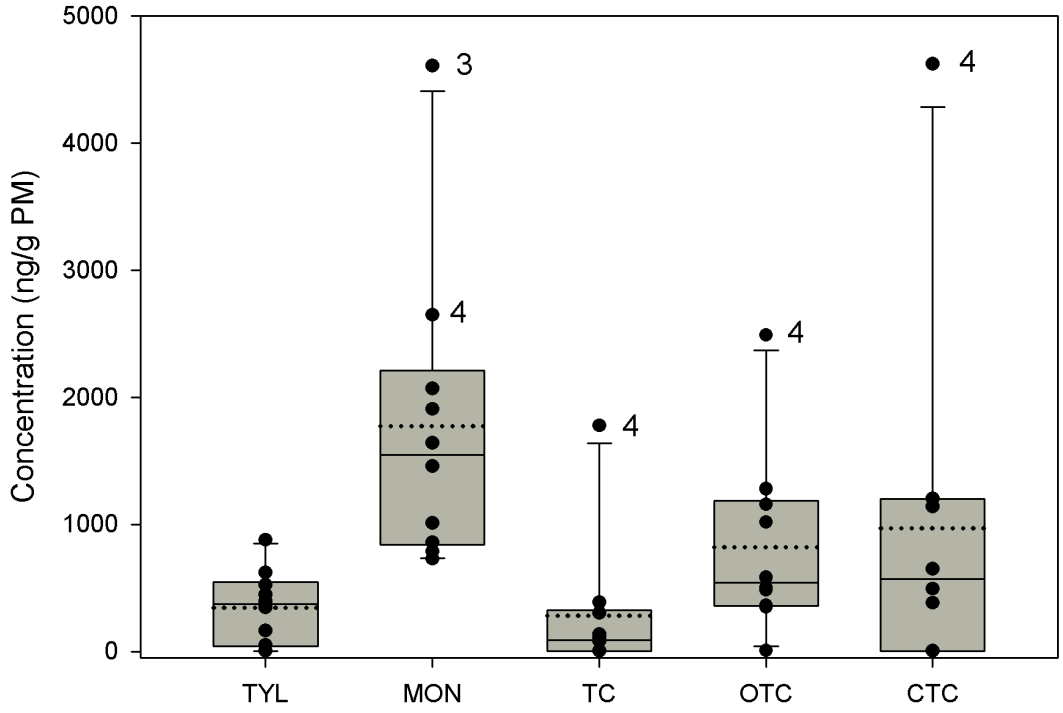

5000
4000
3000
2000
1000
0
Concentration (ng/g PM)
3
4
4
4
4
TYL
MON
TC
OTC
CTC

Figure 2.

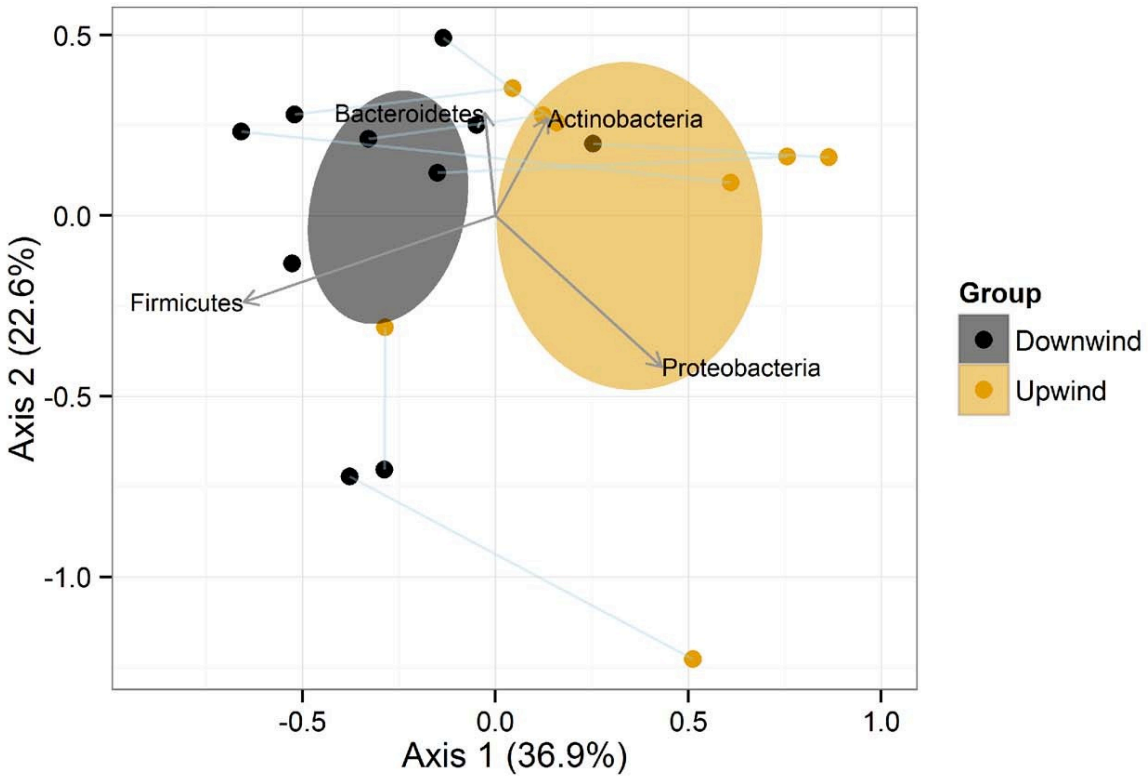
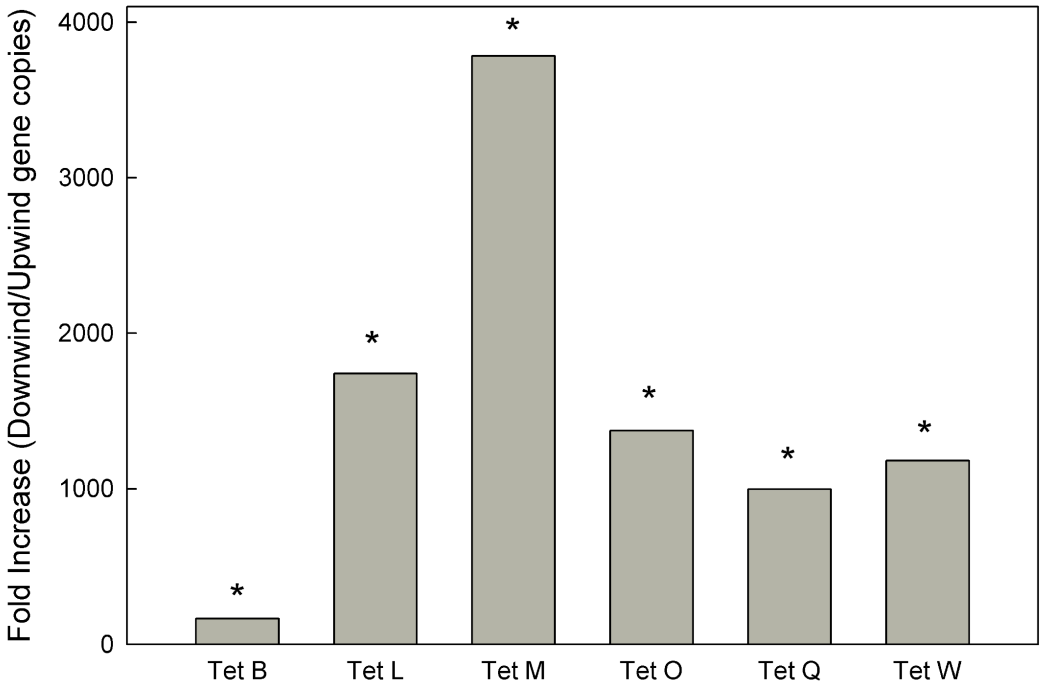

0.5
0.0
-0.5
-1.0
Axis 2 (22.6%)
-0.5
0.0
0.5
1.0
Axis 1 (36.9%)
Bacteroidetes
Actinobacteria
Firmicutes
Proteobacteria
Group
Downwind
Upwind

Figure 3.


Fold Increase (Downwind/Upwind gene copies)
4000
3000
2000
1000
0
*
*
*
*
*
*
Tet B
Tet L
Tet M
Tet O
Tet Q
Tet W