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Food animal transport: A potential source of community exposures to health hazards from industrial farming (CAFOs)

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Summary Use of antimicrobial feed additives in food animal production is associated with selection for drug resistance in bacterial pathogens, which can then be released into the environment through occupational exposures, high volume ventilation of animal houses, and land application of animal wastes. We tested the hypothesis that *current methods of transporting food animals from farms to slaughterhouses may result in pathogen releases and potential exposures of persons in vehicles traveling on the same road.* Air and surface samples were taken from cars driving behind poultry trucks for 17 miles. Air conditioners and fans were turned off and windows fully opened. Background and blank samples were used for quality control. Samples were analyzed for susceptible and drug-resistant strains. Results indicate an increase in the number of total aerobic bacteria including both susceptible and drug-resistant enterococci isolated from air and surface samples, and suggest that food animal transport in open crates introduces a novel route of exposure to harmful microorganisms and may disseminate these pathogens into the general environment. These findings support the need for further exposure characterization, and attention to improving methods of food animal transport, especially in highly trafficked regions of high density farming such as the Delmarva Peninsula.

Abbreviations: IFAP, industrial food animal production; CAFO, concentrated (or confined) animal feeding operation; ARB, antimicrobial-resistant bacteria; PTV, poultry transport vehicles; AHB, aerobic heterotrophic bacteria; PBS, phosphate buffered saline; LOD, limit of detection; cfu, colony forming units.

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Introduction

The industrial food animal production (IFAP) environment, of which some operations (depending upon size) are also known as concentrated animal feeding operations (CAFOs), is a well characterized source of antibiotic-resistant pathogenic bacteria, with documented exposures to human populations via multiple pathways [1—4]. Our research in this area has characterized both food and environmental pathways of exposure to antimicrobial-resistant bacteria (ARB) in the context of broiler poultry production on the Delmarva Peninsula, one of the major production areas in the US [5—7].

The purpose of this project was to investigate a hitherto unstudied route of potential community exposures to ARBs from poultry production: the transport of live flocks from poultry houses to processing plants. Food animals are customarily transported from CAFOs to slaughterhouses in pens, open cages, or crates stacked on flat bed trailers or in a trailer with little or no containment. These methods provide no barrier to pathogens into the environment. Transport cages are known to become highly contaminated with feces and bacteria during the process of transport [8—13]. Ramabu et al. [14], in a study of *Campylobacter* contamination within the broiler poultry production environment, found that after transport, poultry crates were contaminated at a rate of 75%, while 47% of truck beds were also contaminated. This phenomenon has been associated with the contamination of flocks as they enter the processing plant [15—17].

This potential route of environmental release is of public health importance to both local and regional communities. In the Delmarva Peninsula, the opportunities for broader population exposure are considerable, since during the summer months hundreds of thousands of vehicles cross through the same roads trafficked by poultry transport vehicles (PTVs) in order to reach major recreation areas in all three states. *The specific aim of this project was to test the hypothesis that persons riding in passenger cars behind live broiler PTVs are at risk of exposure to antibiotic-resistant bacteria originating from uncontrolled releases of feces and other materials from the open crates of PTV.*

Methods

Feasibility study

Prior to undertaking this study, we first validated methods of sample collection in cars through trial runs in Baltimore City and in the Delaware, Maryland and Virginia (Delmarva) peninsula. We ran several experimental runs in the same or nearby locations as the routes utilized by PTVs. After confirming methods for air and surface sampling, we conducted three environmental sampling trips, from June through October 2007.

Sampling site and collection

This study was conducted on the Delmarva Peninsula, a region with the highest density of broiler chickens per farm acre in the United States, in which several major poultry processing plants are also located [18] (see Fig. 1; area of sampling is indicated by the dashed oval). The route of sampling (route 13) was selected because it is the only access road from farms in Maryland to processing plants in Virginia. We chose PTVs traveling to a processing plant in Accomack, VA, since this facility was located approximately 17 miles (~20 min) south from the start point for this study, which

Figure 1 Delmarva peninsula, with the sampling area indicated by the dashed oval. Map of the Delmarva Peninsula provided by: www.worldatlas.com.

maximized the time for following each PTV. Each sampling run was carried out by a four door passenger vehicle traveling from the starting point to the plant. Briefly, each vehicle followed a truck (2−3 car lengths distance behind) for 17 miles until reaching the processing plant. Air conditioners and fans were turned off and all four windows were fully opened during each sampling run. Three separate air sampling events were conducted on June 20 2007, August 14 2007, and October 18 2007, consisting of 1 run, 6 runs, and 3 runs respectively, for a total of 10 runs. Surface sampling was conducted only on the August and October runs, for a total of 5 runs. Start and end times, temperature and relative humidity were recorded for each run.

Air samples were collected using two methods: a sterile all-glass sampler (BioSampler®, SKC Inc) with 20 ml sterile 1× PBS as collection media and 25 mm gelatin filters (Sartorius, Germany) on sterile open-faced cassettes (Derlin 1107, Pall Life Sciences, Ann Arbor, MI). The BioSampler® collected air samples at a calibrated flow rate of 12.5 L/min, while the open-faced filter had a calibrated flow rate of 4 L/min. The BioSampler was placed in the center of the back seat, with the air intake facing directly to the front. Air was drawn with a vacuum pump (VP0435A, MEDO USA) connected through a power inverter (Pwrinv 400 W, AIMS Corp., Taiwan, ROC) to the vehicle's power. The filter holder was clipped to the side of the driver's head rest at a slightly downward angle (to simulate normal breathing and avoid direct impaction). Both samplers are designed to collect bioaerosols larger than 0.3 μm in diameter. At the termination of each run (within 10 min) the filters were aseptically placed in tubes containing sterile PBS, and the remaining BioSampler® volumes (the air flow caused some evaporation) were recorded and pipetted into sterile tubes.

Surface samples were collected using sterile swabs, wetted with sterile PBS, from two designated surfaces: external, from the outside door handle on the driver's side, and internal: from the top surface of an unopened soda can placed in the cup holder between the driver and front passenger seats. Wipes were collected by wiping for approximately 20 s, and then stirred into sterile PBS in a tube for another 20−30 s.

Blank and background control samples were also collected for both air and surface sampling. Blank air control samples consisted of collection liquid and filters that were inside the car but not exposed to the air. Surface blanks were collected immediately after swiping the target surface with an alcohol swab. Background control air samples were collected during a drive in the same road, in the absence of a PTV, using the same sampling protocol as described above; surface background controls were collected after this control drive. All samples were stored on ice, and processed immediately upon returning to the laboratory (within 8 h).

Bacterial isolation and enumeration

Total aerobic heterotrophic bacteria (AHB) and *Enterococcus* spp. samples were isolated and enumerated using respective agar media, all obtained from Becton Dickinson (Sparks, MD). Samples were analyzed for culturability using standard dilution and spread-plating methods, as well as membrane filtration technique [19]. Briefly, a 1 mL aliquot of each sample was passed through a 47 mm diameter, 0.45 μm pore size cellulose membrane filter (GE Water & Processing Technologies) using a filter funnel and vacuum system. Microorganisms present in the sample are retained on the surface of the filter which is then placed on a nutrient agar plate. In addition, 100 μL aliquots of each sample are plated on nutrient agar. AHB spp. were isolated on tryptic Soy agar, while *Enterococcus* spp. were isolated on enterococcosel agar. All samples were plated in triplicate. Tryptic soy agar plates were incubated at 37 °C for 24 h, while enterococcosel agar plates were incubated at 37 °C for 36 h. Blank and background controls were included in all analyses.

Resulting colonies were counted, and used to determine the concentration of isolated bacteria per cubic meter of air or per square centimeter of surface area as per Eqs. (1) and (2). Enterococcal colonies were indicated by the formation of a black precipitate on enterococcosel agar. Selected presumptive enterococci isolates (Note: previous research on air samples [20] showed that not all isolates growing on this media were *Enterococcus* spp., and therefore we will use the term ''presumptive'') were re-plated on enterococcosel agar and archived in 10% glycerol−tryptic soy broth at −80 °C for subsequent antimicrobial susceptibility testing.

$$\frac{\text{cfu}}{\text{m}^3_{\text{air}}} = \frac{\text{cfu}}{V_{\text{p}}\,(\text{mL}_{\text{liq}})} \times \frac{V_{\text{f}}\,(\text{mL}_{\text{liq}})}{Q_{\text{s}}\,(\text{L}_{\text{air}}/\,\text{min}) \times t_{\text{s}}\,(\text{min}))} \times \frac{1000\,(\text{L}_{\text{air}})}{\text{m}^3_{\text{air}}} \quad (1)$$

$$\frac{\text{cfu}}{\text{cm}^2} = \frac{\text{cfu}}{V_{\text{p}}\,(\text{mL}_{\text{liq}})} \times \frac{V_{\text{f}}\,(\text{mL}_{\text{liq}})}{A_{\text{s}}\,(\text{cm}^2)} \quad (2)$$

where cfu is the colony forming units per plate, V_{p} the volume of liquid plated (mL_{liq}), V_{f} the final volume of liquid from where samples were plated

(mL_{liq}), Q_s the air flow rate (L_{air}/min), T_s the sampling time (min), and A_s is the area swiped (cm^2).

Due to poor recovery of resistant isolates during enterococcal isolation of samples collected from runs 1 and 2 using the protocol mentioned above, we decided to increase the sensitivity of the assay for detection of resistant enterococcal isolates in the samples from run 3 by enriching samples in nutrient broth supplemented with antibiotics at breakpoint concentrations [5]. This strategy optimizes the likelihood of recovering resistant isolates if these are present. Isolates were enriched in enterococcosel broth with the following antibiotics (concentrations): tetracycline (8 μg/ml), vancomycin (16 μg/ml), erythromycin (4 μg/ml), and quinupristin–dalfopristin (2 μg/ml).

Since most of the background samples were below the limit of detection (<LOD), a method LOD was calculated for comparison purposes. This is determined by the minimum number of colony forming units (cfu) per plate, following EPA SW 846 [21]. Eqs. (3) and (4) show the LOD for each method, which was used for graphically representing our data, and where V_s is the volume of total sample, V_p is the volume of sample plated, A_s is the total surface area swiped, and V_a is the volume of air sampled.

$$\text{LOD surface}\left(\frac{\text{cfu}}{\text{cm}^2}\right) = \frac{5\,\text{cfu}}{V_p\,(\text{mL}_{liq})} \times \frac{V_s\,(\text{mL}_{liq})}{\text{swab}} \times \frac{1\,\text{swab}}{A_s\,(\text{cm}^2)} \quad (3)$$

$$\text{LOD air}\left(\frac{\text{cfu}}{\text{m}^3_{air}}\right) = \frac{5\,\text{cfu}}{V_p\,(\text{mL}_{liq})} \times \frac{V_s\,(\text{mL}_{liq})}{\text{sample}} \times \frac{\text{sample}}{V_a\,(\text{m}^3_{air})} \quad (4)$$

Antibiotic susceptibility testing

The disk diffusion method [22] was used to assess the susceptibility of presumptive enterococci isolates ($n = 104$) to eight different antimicrobial drugs. The antimicrobial drugs (including concentrations) used to test for susceptibility were selected based on our previous studies in the broiler poultry environment: penicillin (10 IU), erythromycin (15 μg), virginiamycin [streptogramin A and B combination] (15 μg), vancomycin (30 μg), streptomycin (300 μg), gentamicin (120 μg), ciprofloxacin (5 μg), and tetracycline (30 μg). All drug-impregnated disks were obtained from Becton Dickinson (Sparks, MD).

To perform the disk diffusion test, archived enterococcal isolates were grown on Tryptic soy agar at 37 °C under aerobic conditions. After 24 h, each bacterial sample was suspended in 4 mL Mueller–Hinton broth with a sterile inoculating loop and adjusted to a 0.5 McFarland standard using a Vitek colorimeter (Hach, Loveland, CO). Using a sterile cotton swab, a standard lawn of each sample was created on Mueller–Hinton agar plates. This was followed by dispensing the antimicrobial disks on each plate providing 33 mm distance between disk centers using a disk dispenser obtained from Becton Dickinson (Sparks, MD). Plates were incubated at 37 °C for 24 h.

The zones of inhibition created by exposure to each antimicrobial drug were measured using a millimeter ruler. The following zone diameters (in mm) correspond to resistance, intermediacy, and susceptibility to respective antibiotics: penicillin [≤14, −, ≥15]; erythromycin [≤13, 14–22, ≥23]; virginiamycin [≤15, 16–18, ≥19]; vancomycin [≤14, 15–16, ≥17]; streptomycin [≤6, 7–9, ≥10]; gentamicin [≤6, 7–9, ≥10]; ciprofloxacin [≤15, 16–20, ≥21]; and tetracycline [≤14, 15–18, ≥19] [22].

Statistical analysis

Statistical significance was considered at $\alpha = 0.05$ level, and tested using STATA® (College Station, TX). Differences between the air samples ($N = 9$) and their respective backgrounds ($N = 4$) were tested using two-sample t-tests with equal variances. Differences between the two air samplers were tested with paired t-tests. Differences between the swab samples ($N = 5$ for internal and 3 for external) and their respective controls ($N = 4$ for internal and 3 for external) were determined with Wilcoxon rank-sum non-parametric tests, due to small sample size.

Results

Enumeration of AHB and enterococci from air and surface samples

Results are presented as concentrations in comparison to background. Background for all methods was <5 cfu/plate. This count was used to establish the LOD for each method.

Based on Eqs. (3) and (4), LOD for the interior wipe = 1.25 cfu/cm^2; LOD for the exterior wipe = 0.25 cfu/cm^2; LOD for the BioSampler samples = 400 cfu/m^3, and LOD for the filter samples = 500 cfu/m^3.

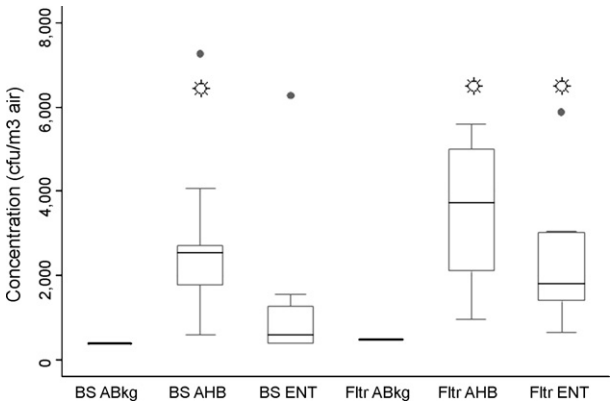

Figure 2 Increased levels of airborne AHB (aerobic heterotrophic bacteria) and presumptive enterococci (ENT) as compared to background levels from both BioSampler (BS) and gelatin filter (Fltr). Data for all 10 runs, June 20, August 14, October 18, 2007. ABkg = Air Background Control. The box extends from the 25th to the 75th percentile. The line across the box represents the median value. The whiskers extend to the most extreme observations that are within 1.5 times the percentiles. Points outside this range represent outliers. ☼ : statistically significant from background.

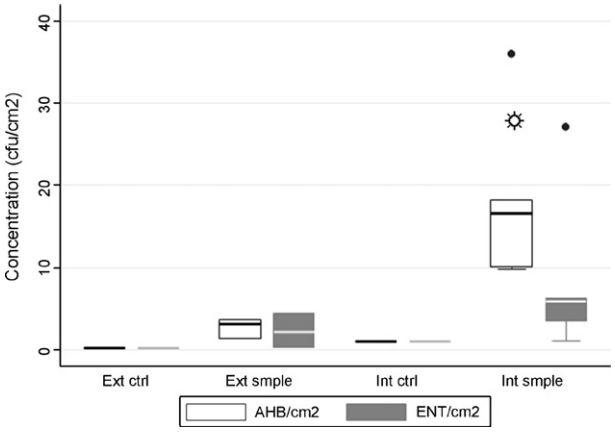

Figure 3 Increased levels of AHB (aerobic heterotrophic bacteria) and presumptive enterococci (ENT) from surface samples as compared to background. External control (Ext ctrl) and Internal control (Int ctrl) are background controls (not following poultry transport vehicles (PTV)). Internal/External sample represent surface samples (following PTV) in cfu/cm^2. $N = 5$ for the internal samples, $N = 4$ for internal controls, and $N = 3$ for external controls and samples. The box extends from the 25th to the 75th percentile. The line across the box represents the median value. The whiskers extend to the most extreme observations that are within 1.5 times the percentiles. Points outside this range represent outliers. ☼ : statistically significant from background; ● : data outlier.

Air samples

Data for all 10 runs are presented in Fig. 2. Mean concentrations of airborne AHB collected while driving behind PTVs were 2.8×10^3 cfu/m^3 for BioSamplers and 3.6×10^3 cfu/m^3 for the filters, both statistically higher than background controls (all background controls were <LOD) ($p < 0.05$). Average concentrations of presumptive airborne enterococci for the control runs were <LOD, with concentrations of 1.3×10^3 cfu/m^3 from the BioSampler ($p > 0.05$) and 2.3×10^3 from the filter sampler ($p < 0.01$).

Surface samples

Interior and exterior wipe samples were positive after driving behind PTVs. Background levels for AHB and presumptive enterococci were below the limit of detection (<1.25 and <0.25 cfu/cm^2 for both interior and exterior surfaces respectively) on both dates. Average levels on the interior surface after driving behind PTV were 18 cfu/cm^2 for AHB ($p < 0.05$) and 9 cfu/cm^2 for presumptive enterococci ($p > 0.05$). Average external AHB and presumptive enterococci levels were 2.8 and 2.3 cfu/cm^2, respectively, both higher than background ($p > 0.05$). All samples were above the limit of detection. Data are presented in Fig. 3.

Antibiotic-resistant enterococci

Tetracycline, erythromycin, quinupristin/dalfopristin, and high level streptomycin resistance phenotypes were detected among isolated presumptive *Enterococcus* spp. ($n = 24$ out of 96, 25%) recovered from airborne and surface samples after traveling behind poultry transport vehicles. No resistant isolates were detected in background samples. Of the 24 resistant isolates, 62.5% were resistant to tetracycline (Tet^r), 41.7% were resistant to erythromycin (Ery^r), one isolate was resistant to quinupristin/dalfopristin and one isolate was resistant to high level concentrations of streptomycin. Three of the 24 isolates were resistant to more than one antibiotic. There was no resistance to vancomycin detected within any of the isolates (Fig. 4).

Discussion

Increased concentrations of AHB and presumptive enterococci were isolated from the air and internal/external surfaces of cars traveling behind PTVs. This confirms the possibility of major pathogen dispersal via this route, and highlights the

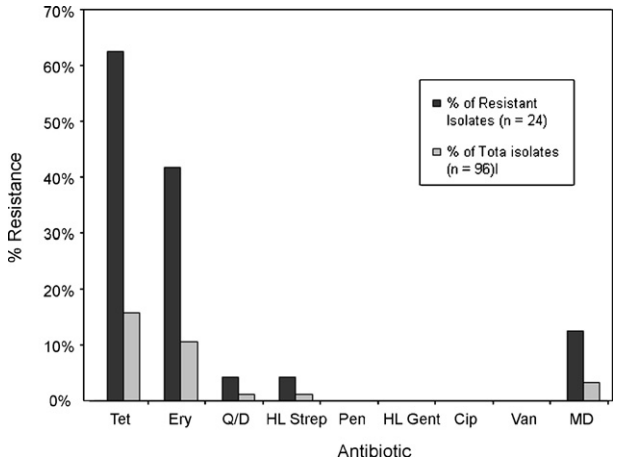

Figure 4 Phenotypic profile for resistant isolates. Percentage of resistant and total isolates resistant to individual antibiotics and multiple antibiotics. Tet: tetracycline, Ery: erythromycin, Q/D: quinupristin/dalfopristin, HL Strep: high level streptomycin, Pen: penicillin, HL Gent: high level gentamicin, Cip: ciprofloxacin, Van: vancomycin, MD: multi-drug.

importance of better containment of animals and waste. Surface samples indicate that airborne bacteria, including ARB strains, can deposit on surfaces and remain viable for up to 20 min. Twelve presumptive enterococci isolates were found to be resistant to three antimicrobial drugs of human importance: tetracycline, erythromycin, and quinupristin/dalfopristin. These three drugs are all approved for use in broiler poultry [23]. These data are consistent with previous research by us and others that has demonstrated associations between antibiotic use in food animal production and the presence of microbial antibiotic resistance [3,5]. Also of importance, we detected no evidence for vancomycin resistance, consistent with other studies in the food animal environment conducted by us and by Chapin et al. [20]. Since vancomycin analogs have never been used in US food animal production [24], this finding supports the inference that the source of ARB in our samples was not likely to be from humans.

Previous reports have revealed heightened risks of ARB exposure to the public and occupational workers through consumption and handling of raw, improperly cooked or live poultry [6]. Water and air sources near and within animal facilities have also been implicated as sources of exposure to drug-resistant bacteria [20,25,26]. Currently, there are no data on air releases from poultry houses. We and others have also reported that workers handling live poultry are at increased risk of exposure to ARB [6,7]. The results from this study indicate that the current practice of transporting poultry (and very likely other food animals) may serve as another route of exposure that requires attention. Populations at increased risk to this exposure pathway include motorists and others traveling behind PTV, as well as neighborhood populations along the PTV path, which likely include susceptible populations such as children and elderly people.

This study has also served to validate the use of gelatin filters for sampling biological aerosols inside a moving vehicle. Gelatin filter data were not statistically different ($p > 0.1$) from the widely used and validated BioSampler [27]. This is a significant finding, since collection of bioaerosols onto filters presents advantages over liquid samplers because of their portability and ease of use. The use of membrane filters has traditionally been problematic due to low survival efficiency caused by cell desiccation and poor microbial recovery from the filters [28]. Gelatin filters offer a moist surface conducive to cell viability and the ability to dissolve completely within the diluent, which precludes the need for cell extraction from the filter.

This first study is limited in size and scope. Moreover, during the conduct of the runs we were aware of several factors that may contribute to variability in results. Weather, including temperature, humidity, and wind direction/velocity, may affect levels of bacteria in air and impact on cars [29]. The speed and distance between PTVs and receiver vehicles, which could not be controlled, also varied among vehicles and runs. Given the empirical nature of this sampling, it is not possible to control all these variables and thus substantially more sampling will be required to more precisely estimate risks of exposure, and to support generalization to other poultry producing areas.

Despite the abovementioned limitations, all but one of the air samples and inside surface swipes yielded statistically elevated bacteria from background, and ARB strains were isolated from both air and surface samples. These data are a strong indication that PTVs are a likely route of exposure to antibiotic-resistant bacteria. These findings support the need for further exposure characterization, and attention to improving methods of food animal transport, especially in highly trafficked regions of high density farming such as the Delmarva Peninsula.

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