

Exposure to Pathogens Among Workers in a Poultry Slaughter and Processing Plant

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Background Working conditions in poultry slaughter/processing plants may expose workers to zoonotic pathogens. We explored exposure to pathogens among poultry slaughter/processing plant workers including job duties as risk factors.

Methods We collected questionnaire data on job duties and nasal swabs from 110 workers at one plant in South Carolina. Swabs were tested for *Staphylococcus aureus* and gram-negative organisms. Isolates were screened for antimicrobial susceptibility.

Results There was no differences in prevalence of *S. aureus* carriage based on job duties. As compared with office or packing workers, the adjusted odds of GNO carriage was 6.29 times (95% CI: 1.43, 27.71) higher in slaughter or carcass processing workers and 5.94 times (95% CI: 0.94, 37.50) higher in cleaning or maintenance workers.

Conclusions Poultry processing plant workers may have increased exposure to GNOs, depending on job duties. Am. J. Ind. Med. 59:453–464, 2016. © 2016 Wiley Periodicals, Inc.

KEY WORDS: occupational exposure; poultry plants; zoonotic pathogens; *Staphylococcus aureus*; MRSA; gram-negative pathogens

INTRODUCTION

Exposures of workers to zoonotic pathogens occur throughout the production of livestock and poultry for consumer products, from the farm to the fork, including animal confinement houses, slaughter and processing plants, as well as during food preparation and consumption. The use of antimicrobial drugs as feed additives in food animal production contributes to the risks of drug resistance in these pathogens [Silbergeld et al., 2008].

Among occupational groups at risk, recent studies by us and others have examined exposures of workers in food animal slaughter/processing plants, where large numbers of animals are processed [Mulders et al., 2010; Castillo Neyra et al., 2014; CDC NIOSH, 2014]. In poultry slaughterhouse workspaces, prevalent *Campylobacter* contamination has been reported [Johnsen et al., 2006] along with outbreaks of campylobacteriosis among the workers [de Perio et al., 2013]. Workers in food animal slaughter/processing plants are at risk of exposure to drug resistant strains of enterococci, *Escherichia coli*, and *Staphylococcus aureus* [van den Bogaard et al., 2002; Thorsteinsdottir et al., 2010; Wendlandt et al., 2013]. In some studies, the resistance patterns in strains isolated from workers have been matched phenotypically with isolates from poultry carcasses in the plant [Thorsteinsdottir et al., 2010] and in other studies genotyping methods have been used to further define exposures in workers as compared to referent groups [Wendlandt et al 2013; Castillo Neyra et al., 2014]. Most of these studies have been reported from the EU; relatively fewer studies have investigated US workers within this industry and not all studies have assessed job duties as risk factors associated with zoonotic pathogen exposure.

In order to better understand the extent to which US poultry slaughter/processing plant workers are exposed to

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bacterial pathogens, this exploratory study aimed to characterize nasal carriage of a subset of bacterial pathogens among workers in a US poultry slaughter/processing plant. We also tested (post hoc) the hypothesis that higher prevalence of nasal gram negative organism (GNO) carriage would be associated with more intense occupational contact with poultry, a potential source of exposure to these organisms. This was done by measuring the prevalence of nasal carriage of these pathogens, assessing the antimicrobial susceptibility of the detected pathogens, and analyzing (post hoc) the association of the nature/extent of occupational poultry contact (as approximated by categorizations of workers' job duties within the plant) with nasal carriage of GNOs.

METHODS

Study Design and Subject Recruitment

We conducted a cross-sectional cohort study of workers at the Columbia Farms broiler poultry slaughter/processing plant in Columbia, South Carolina over two enrollment rounds in November 2013 (3 days) and April 2014 (1 day). The unionized workforce at this plant included approximately 635 workers, out of approximately 775 total employees. Based on information from union officials, there were no differences between the non-unionized and unionized workers with the same job positions in terms of job duties within work assignment in the plant. Plant workers all reported living close to the city. We enrolled workers with the assistance of the United Food and Commercial Workers International Union (UFCW), which represents this workforce. Prior to enrollment, we conducted formative research to understand the workflow and job duties within the plant. We also developed and pilot tested our English language questionnaire (according to UFCW, no Spanish-only speaking workers were employed following a sweep by Immigration and Customs Enforcement) on six of the unionized workers to improve its accuracy, clarity, and consistency. Through notices and personal communications from the local UFCW representatives and shop stewards, workers from all shifts at the plant were informed of the scheduled enrollment times and location (a local church within walking distance of the plant). Before study initiation, the local union informed Columbia Farms of our proposed study. The study was reviewed and approved by the Johns Hopkins School of Public Health Institutional Review Board.

Subject Enrollment

The recruitment target for this exploratory study was 110 participants. Inclusion criteria were age ≥ 18 years; current employment at Columbia Farms; ability to

understand an orally administered questionnaire in English; and willingness to contribute a nasal swab. Prior to any data collection, participants confirmed their consent to a form that was read to them. Upon completion of enrollment and data collection, each participant received a gift card to a local store for \$25 to compensate for their time.

Data Collection and Biological Sampling

All interviewers first reviewed the pretested questionnaire together and received training for consistent questionnaire administration. They then used the questionnaire, in one-on-one interviews, to collect participant data, including demographics, occupational duties and characteristics, contact with animals outside of the plant, recent health history (including injuries, infections, antibiotic usage, health care contact), and typical diet.

After completing the questionnaire, a biological sample was collected from both nares of each participant by trained researchers wearing sterile gloves and using aseptic techniques and methods based on CDC recommendations (CDC NHANES [2015], for an example see Giesinger Medical Laboratories instructions [2015]) with a dual swab with BBLTM CultureSwabTM Plus dual swab (BD Diagnostic Systems). Each rayon-tipped swab applicator was then placed into its plastic tube containing Amies gel without charcoal and this tube was then re-inserted into the original sterile peel pack. After each sample was taken, the researchers removed gloves, used hand sanitizer, and regloved with a new sterile pair of gloves. Nasal sampling and sample management was conducted in a separate area at the enrollment site set up solely for this part of the study. All swab samples were delivered to our laboratory at Johns Hopkins University by express courier service within 48 hr of collection.

No individual identification information was collected; participants were coded numerically upon entry into the study and the same code was used on both questionnaires and swab samples.

Microbiological Analyses

Upon arrival at our laboratory, nasal swabs were immediately transported at room temperature to the Johns Hopkins Hospital Clinical Microbiology Laboratory, where they were processed within 72 hr of collection. All swabs were inoculated onto BBLTM CHROMagarTM Staph aureus (BD Diagnostic Systems) as well as BBLTM TrypticaseTM Soy agar with 5% sheep blood (TSA II) (BD Diagnostic Systems) as described previously [Flayhart et al., 2004]. Plates were incubated in a non-CO₂ incubator at 37°C and read at 24 and 48 hr according to manufacturer's recommendations.

S. aureus latex agglutination (Pro-Lab Diagnostics, Ontario Canada) was performed on any mauve colored colony, similar to Flayhart et al. [2004]. Any latex positive isolates were identified as *S. aureus* and sub-cultured on TSA II agar to isolate pure colonies. In processing the samples from participants recruited on the first day of the study, the clinical microbiology laboratory noted that many samples were positive for gram-negative organisms (GNOs); consequently for all 90 participants enrolled after that date, in addition to testing for *S. aureus*, cultured GNOs were recovered from the initial TSA II plates and streaked on new TSA II plates to obtain pure colonies. Gram-negative species were characterized by 16S rRNA gene amplification and sequencing as detailed below. All isolates were transferred into 30% glycerol and frozen at -80°C. One *S. aureus* isolate per individual, and up to two GNOs per individual (from the most prevalent morphologies) were further analyzed in our laboratory.

Molecular Analyses

DNA was extracted from each isolate using DNeasy Blood & Tissue kit (Qiagen) according to the manufacturer's protocol for gram-positive or gram-negative bacteria. A multiplex PCR assay was performed for all *S. aureus* isolates to amplify their 16S rRNA, *nuc*, and *mecA* genes [Poulsen et al., 2003]. Isolates positive for *nuc* were confirmed as *S. aureus*; isolates positive for both *nuc* and *mecA* were classified as genotypic MRSA (methicillin resistant *S. aureus*). For identification of GNOs, we amplified their nearly complete 16S rRNA genes using universal eubacterial primers 27f and 1492r [Weisburg et al., 1991]. PCR products were verified by gel electrophoresis and sequenced on both strands using the same primers on a 3730xl DNA Analyzer (Applied Biosystems). After assembly using SeqMan Pro (DNASTAR), the 16S rRNA gene sequences were queried against records in the GenBank using BLASTn and also analyzed by the Ribosomal Database Project Classifier program [Cole et al., 2009].

Antimicrobial Susceptibility Testing

All *S. aureus* isolates and the GNO isolates belonging to the five most frequently detected genera (*Acinetobacter*, *Citrobacter*, *Enterobacter*, *Proteus*, *Pseudomonas*) were tested for antimicrobial susceptibility using the micro-dilution method with BBL Mueller Hinton II broth (cation-adjusted; BD Diagnostic Systems), according to the protocols of the Clinical and Laboratory Standards Institute [CLSI, 2012]. Briefly, isolates were first regrown on Mueller Hinton agar and then direct colony suspensions were inoculated in duplicate into 96-well microtiter plates with each series of wells containing one drug in a twofold dilution

series (see Table S1). *S. aureus* isolates were tested for susceptibility to drugs used in poultry production [Silbergeld et al., 2008] as well as drugs of clinical importance: cefoxitin, ciprofloxacin, clindamycin, erythromycin, gentamicin, virginiamycin, tetracycline, and trimethoprim-sulfamethoxazole. GNOs were examined for susceptibility to a range of drugs, depending upon the species being tested: ampicillin, ceftazidime, ciprofloxacin, ertapenem, gentamicin, meropenem, piperacillin-tazobactam, tetracycline, and trimethoprim-sulfamethoxazole. These drugs were chosen, with consultation with the Johns Hopkins Hospital Clinical Microbiology Laboratory, based on the CLSI recommendations (2013) and are currently included in commercial systems for clinical settings [Snyder et al., 2008].

For each isolate and drug, minimum inhibitory concentration values were determined visually after 16–20 hr of incubation at 35°C as the lowest concentration of a drug that inhibited all apparent bacterial growth. Isolates were classified as susceptible, intermediate, or resistant to antimicrobials according to CLSI standards (2013). MRSA was defined phenotypically as resistance to cefoxitin and genotypically by the presence of *nuc* and *mecA* genes as described above. *S. aureus* ATCC 29,213 and 43,300, *E. coli* ATCC 25,922, and *Pseudomonas aeruginosa* ATCC 27,853 were included in each batch of testing for quality control [CLSI, 2012].

Statistical Analyses

Based on self-reported department of employment and open-ended description of work duties, participants were initially assigned to five job categories: (i) handling live chickens (n = 7); (ii) processing (which includes evisceration, cutting, deboning, and sorting duties along the carcass processing line, as well as supervising and Quality Control duties in these work areas) (n = 55); (iii) maintenance/cleaning (n = 21); (iv) packing poultry products (n = 17); and (v) others (shipping, box-making, or office activities) (n = 10). The five job categories were defined based on estimated exposure intensity to poultry, as inferred from the questionnaire via either direct contact or inhalation of bioaerosols, which has been suggested by other studies [Whyte et al., 2001; Lues et al., 2007; Liang et al., 2013]. Job categorization was further guided by information about the plant layout, gained from on-site observation, and conversations with local UFCW workers. When self-reported questionnaire responses identified workers with multiple job duties fitting different job categories, the assigned job category was based on the type of work with the highest estimated intensity of exposure to live poultry, poultry carcasses, or bioaerosols. Due to small category sizes, for analysis these initial five categories were further collapsed into three categories, based on exposure similarity,

namely “pre-slaughter/processing,” “maintenance/cleaning,” and “packing/other.” Prior to collapsing, similarity in microbiological outcomes was also checked. This categorization process is further detailed in the Supplemental Material (SM).

A flow chart for the study is presented in Figure 1. The prevalence of nasal *S. aureus* was determined for all 110 participants and the prevalence of GNOs was determined for the sub-group of 90 participants enrolled after the first day of enrollment. In addition, for *S. aureus* and the most frequently detected GNOs (*Acinetobacter*, *Citrobacter*, *Enterobacter*, *Proteus*, and *Pseudomonas*) the proportion of non-susceptible isolates (intermediate or resistant by CLSI standards) was determined and patterns of antimicrobial resistance assessed.

The distributions of variables representing demographic and other relevant information were examined and compared among the job categories, using Fisher’s exact test for categorical variables and one-way analysis of variance (with the Bonferroni correction to account for multiple comparisons) for continuous variables. This was done for the full study cohort, and for the sub-group assessed for nasal GNOs.

Since relatively few participants carried *S. aureus* or MRSA, further analyses of the data focused on the 90 participants screened for GNOs. The odds of detecting any nasal GNO among the three job categories were compared

using unadjusted and adjusted logistic regression models. Covariates considered for the final adjusted model were age, gender, pet ownership, consistent face mask usage (definition in SM), self-reported antimicrobial usage in the past 6 months (yes/no), working shift (before third shift/shift), and recruitment category. The first five covariates were identified using *a priori* assumptions; working shift was identified based on the plant’s schedule, as live chickens arrive during the first and third shift while plant cleaning occurs during the second shift; and recruitment category was assessed to check for potential unmeasured differences between the recruitment rounds. Gender, self-reported antimicrobial usage, and working shift were selected for the final model due to their observed behavior in this dataset; further details regarding covariate selection are described in SM. A sensitivity analysis was performed to check the impact of outliers on the final logistic regression model; alternative logistic regression models were utilized to check the robustness of results to covariate selection decisions. These models are further described in SM. The small sample size precluded additional covariates in the final model.

Statistical and graphical analyses were performed using Stata version 13.1 (StataCorp, College Station, TX), and R version 3.0.3 (R Foundation for Statistical Computing, Vienna, Austria).

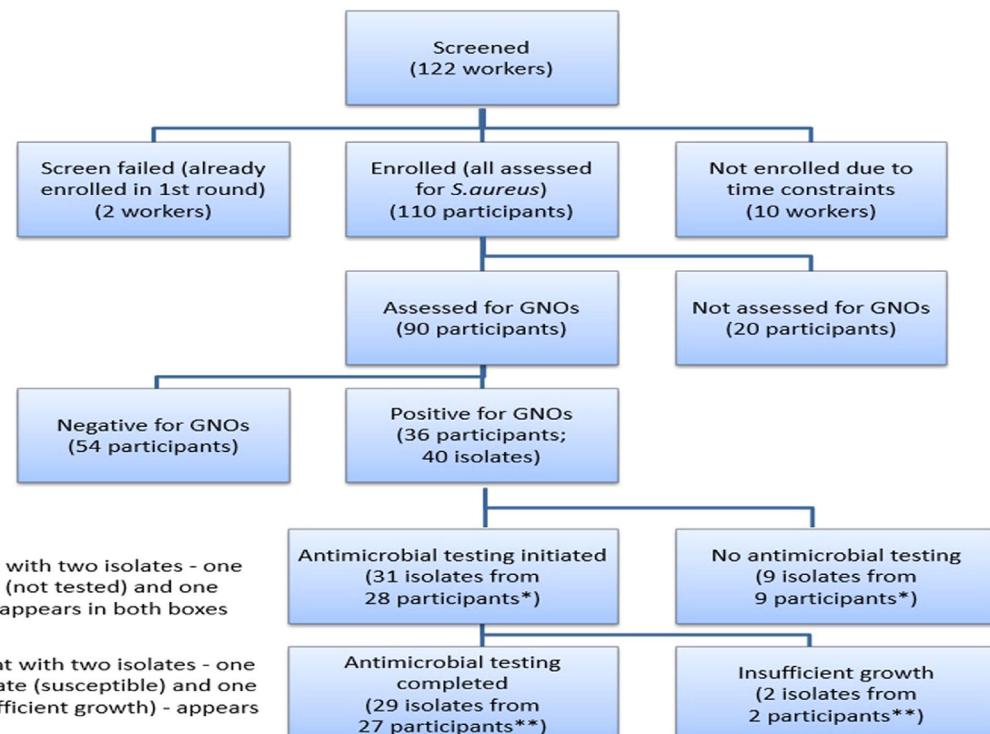


FIGURE 1. Flowchart of study procedures. As detailed in the methods, a subset of enrolled participants were not assessed for nasal GNOs. Of the detected GNOs, 31 isolates from the five most frequently detected genera underwent antimicrobial susceptibility testing. Note that in four participants, multiple morphologically distinct isolates (two per participant) were identified.

RESULTS

Study Population and Job Categories

A total of 110 participants from one poultry slaughter and processing plant were enrolled in this study, including workers who reported handling live poultry, carcass evisceration, cutting and processing fresh carcasses, cleanup and maintenance, packing, shipping, and office work. These participants represented approximately 17.3% of the plant's unionized workforce at the time. The majority of the participants were African American (87.3%) and male (64.5%) (Table I).

Using the three collapsed job categories, the pre-slaughter/processing category had the highest proportion of African Americans (93.6%); the maintenance/cleaning category had the lowest proportion (71.4%) ($P = 0.027$, comparing all three groups) (Table I). The differences in gender distributions within the job categories were of borderline statistical significance ($P = 0.054$), with most female participants in the pre-slaughter/processing category (45.2%) and fewest in the maintenance/cleaning (19.1%) category. Participants in the packing/other category were the youngest and those in the maintenance/cleaning category were the oldest (mean \pm SD: 40.6 ± 10.2 vs. 50.0 ± 11.9 , comparing all three groups $P < 0.01$). Consistent face mask usage was most frequently reported by participants in the pre-slaughter/processing category (38.7%) and least frequently reported by those in the packing/other category (11.1%) ($P = 0.019$). Among the pre-slaughter/processing participants, 15/28 (53.6%) of the women, but only 9/34 (26.5%) of the men reported consistent face mask usage ($P = 0.038$). In the other two job categories, there were no statistically significant differences by gender in reported consistent face mask usage. The maintenance/cleaning category had the highest proportion of participants working before the third shift (85.7%) and the packing/other category had the lowest proportion (18.5%) (comparing all three groups $P < 0.001$), consistent with the aforementioned plant schedule. Pet ownership was most frequently reported among participants in the maintenance/cleaning category (52.4%) and least frequently reported among those in the pre-slaughter/processing category (22.6%) (comparing all three groups $P = 0.016$). No statistically significant differences were observed among job categories for any of the other covariates measured. Thirteen participants in the cohort (11.8%) reported second jobs; however, their descriptions were not indicative of high risk of GNOs or *S. aureus* exposure. In addition to Table I showing variable distributions for all 110 participants, Table S4 summarizes the distribution of these variables for the sub-group of 90 participants also assessed for GNOs (those enrolled after the first day of enrollment).

Prevalence of *S. aureus*, Non-Susceptible *S. aureus*, and MRSA

We assessed *S. aureus* carriage in all 110 participants, among whom the overall prevalence of nasal carriage of *S. aureus* was 14.6% (16/110) (Table I). Although the observed prevalence was slightly higher in participants in the pre-slaughter/processing category (11/62) than in the other categories, this difference was not significant.

Antimicrobial susceptibility was determined for all *S. aureus* isolates from 16 workers. Seven isolates (43.8%) were non-susceptible to at least one of the drugs tested. Of those, one worker in the pre-slaughter/processing category, whose self-reported job description entailed removing poultry carcasses from the processing line, carried MRSA (classified phenotypically and genotypically).

Prevalence of Gram-Negative Organisms (GNOs) and Non-Susceptible GNOs

We assessed nasal GNO carriage in only those 90 participants enrolled after the first day of enrollment based upon initial reports from the clinical microbiological laboratory of high rates of overgrowth by GNOs during culture of samples collected on the first day (flow chart of sample analysis shown in Fig. 1). The packing/other category had the lowest proportion of participants assessed for nasal GNOs (63.0%); the pre-slaughter/processing category had the highest proportion (93.6%) assessed (comparing all three groups $P = 0.001$) (Table I). Among the 90 tested participants, 36 were positive for nasal GNOs (40.0%). By job category, 26/58 (44.8%) of participants in the pre-slaughter/processing category, 7/15 (46.7%) of participants in the maintenance/cleaning category, and 3/17 (17.6%) of participants in the packing/other category were positive for nasal GNOs (Table II).

Forty GNOs were obtained from these 36 participants, in most cases one isolate per individual. From 4/36 participants, two morphologically distinct GNOs were obtained. These four individuals were all in the pre-slaughter/processing category. *Acinetobacter* (11/40) was the most prevalent genus observed in our samples, followed by *Citrobacter* (7/40) and *Pseudomonas* (5/40). Less prevalent genera were: *Proteus* (4/40), *Enterobacter* (4/40), *Chryseobacterium* (3/40), *Klebsiella* (2/40), *Moraxella* (1/40), *Pantoea* (1/40), *Serratia* (1/40), and *Wautersiella* (1/40) (Fig. 2 and Table S2). Among these, CDC has issued warnings regarding increasingly prevalent antimicrobial resistance among *Acinetobacter* spp. and *Enterobacteriaceae*, as well as *P. aeruginosa* [CDC, 2013]. We observed 30 isolates that were either *Acinetobacter* spp. or *Enterobacteriaceae* (Table S2). Twenty-three of these 30 isolates were from participants in the pre-slaughter/processing category.

TABLE I. Cohort Population Characteristics by Job Categories

Characteristic	Total n = 110	Pre-slaughter/processing n = 62	Maintenance/cleaning n = 21	Packing/other n = 27	P-value ^a
Demographics					
Age (mean ± SD)	42.9 ± 11.79	41.5 ± 11.70	50.0 ± 11.85	40.6 ± 10.16	0.008
Female gender	39 (35.45%)	28 (45.16%)	4 (19.05%)	7 (25.93%)	0.054
Race/ethnicity					
African-American/black	96 (87.27%)	58 (93.55%)	15 (71.43%)	23 (85.19%)	0.027
Other	14 (12.73%)	4 (6.45%)	6 (28.57%)	4 (14.81%)	
Education					
High school/GED or below	85 (77.27%)	49 (79.03%)	17 (80.95%)	19 (70.37%)	0.672
Beyond high school/GED	25 (22.73%)	13 (20.97%)	4 (19.05%)	8 (29.63%)	
Occupational					
Second job ^b	13 (11.82%)	9 (14.52%)	1 (4.76%)	3 (11.11%)	0.611
Full time shifts (average working day ≥8 hpd)	77 (70.00%)	40 (64.52%)	14 (66.67%)	23 (85.19%)	0.117
Same job duties over the course of the month	86 (78.18%)	46 (74.19%)	18 (85.71%)	22 (81.48%)	0.571
Works before 3rd shift ^c	49 (44.55%)	26 (41.94%)	18 (85.71%)	5 (18.52%)	< 0.001
Round recruited					
Round 1 (Nov 2013)	50 (45.45%)	23 (37.10%)	13 (61.90%)	14 (51.85%)	0.110
Round 2 (Apr 2014)	60 (54.55%)	39 (62.90%)	8 (38.10%)	13 (48.15%)	
Self-reported consistent mask usage ^{d,e}	31 (28.18%)	24 (38.71%)	4 (19.05%)	3 (11.11%)	0.019
Among males	15/71 (21.13%)	9/34 (26.47%)	4/17 (23.53%)	2/20 (10.00%)	
Among females	16/39 (41.03%)	15/28 (53.57%)	0/4	1/7 (14.29%)	
Gender comparison P-value ^a	0.045	0.038	0.546	1.000	
Medical					
Contact with health care in last 6 months ^d	71 (64.55%)	39 (62.90%)	14 (66.67%)	18 (66.67%)	0.927
Use of antibiotics in last 6 months	32 (29.09%)	16 (25.81%)	7 (33.33%)	9 (33.33%)	0.712
MRSA diagnosis in the last year ^f	2 (1.82%)	1 (1.61%)	1 (4.76%)	0	0.405
Household/Community					
Pet owners	37 (33.64%)	14 (22.58%)	11 (52.38%)	12 (44.44%)	0.016
Animal manure contact (outside of work)	3 (2.73%)	1 (1.61%)	2 (9.52%)	0	0.140
Butchered an animal in the last 6 months (outside of work)	4 (3.64%)	4 (6.45%)	0	0	0.385
Lives on a farm or nearby a farm/processing plant ^d	8 (7.27%)	4 (6.45%)	1 (4.76%)	3 (11.11%)	0.609
<i>S. aureus</i> testing					
<i>S. aureus</i>	16 (14.55%)	11 (17.74%)	1 (4.76%)	4 (14.81%)	0.381
Non-susceptible <i>S. aureus</i> ^g	7 (6.36%)	6 (9.68%)	1 (4.76%)	0	0.235
MRSA	1/110 (0.91%)	1/62 (1.61%)	0	0	1.000
GNO testing					
Assessed for nasal GNOs	90 (81.82%)	58 (93.55%)	15 (71.43%)	17 (62.96%)	0.001
Prevalence of nasal GNOs	36/90 (40%)	26/58 (44.83%)	7/15 (46.67%)	3/17 (17.65%)	0.114

With the exception of *S. aureus* and GNO testing, all data are self-reported via the study questionnaire. Percentages are based on the entire study population of 110 workers unless stated otherwise.

^aP-values calculated using Fisher's exact test for categorical variables and one-way analysis of variance test (with the Bonferroni correction for multiple comparisons) for continuous variables.

^bDescriptions of reported second jobs are as follows: "chef; Ft. Jackson, cleaning; hair stylist; kitchen; tire business; custodial supervisor; volunteer cooperative ministry; restaurant; painting; construction; cleaning at University of SC; sale—used tires; [missing]."

^cLive chickens arrive at the plant for the first and the third shift. During the second shift, plant cleaning occurs and no live chickens arrive.

^dSee SM for definition.

^ePlant policy requires workers handling live animals or holding certain quality control positions to wear face masks; face mask usage is optional for other positions.

^fBoth diagnosed from a skin infection or wound.

^gAll non-susceptible to erythromycin, with one also being a MRSA.

TABLE II. Nasal GNO Status, by Job Category and Nasal *S. aureus* Status, of the 90 Participants Enrolled After the First day of Enrollment

Nasal GNO status	Nasal <i>S. aureus</i> status by job category			Total (n = 90)
	Pre-slaughter/processing (n = 58)	Maintenance/cleaning (n = 15)	Packing/other (n = 17)	
Negative	32 (55.2%, 95% CI: 41.5%, 68.3%)	8 (53.3%, 95% CI: 26.6%, 78.7%)	14 (82.4%, 95% CI: 56.6%, 96.2%)	54 (60%, 95% CI: 49.1%, 70.2%)
Positive	26 (44.8%, 95% CI: 31.7–58.4%)	7 (46.7%, 95% CI: 21.5–73.4%)	3 (17.6%, 95% CI: 3.8–43.4%)	36 (40%, 95% CI: 29.8%, 50.9%)

Confidence intervals are 95% exact binomial confidence intervals.

Antimicrobial susceptibility was conducted for 31 isolates from the five most commonly detected GNO genera (*Acinetobacter*, *Citrobacter*, *Enterobacter*, *Proteus*, and *Pseudomonas*). Two isolates, one as *Acinetobacter johnsonii* and one as *Pseudomonas* spp., could not be regrown for testing (Fig. 1). Of the 29 tested isolates, the proportion of isolates non-susceptible to at least one antimicrobial was 9/29 (31.0%), and the proportion of isolates resistant to at least one antimicrobial was 8/29 (27.6%). Four of the 29 isolates (13.8%) were non-susceptible to two of the tested antimicrobials (Fig. 3).

Antimicrobial Resistance Profiles for *S. aureus* and GNOs

S. aureus isolates in this study were susceptible to most of the tested antimicrobials. Seven of the 16 *S. aureus* isolates were non-susceptible to erythromycin; one of the seven was also resistant to cefoxitin.

The four *Proteus* spp. isolates (all *P. mirabilis*) were resistant to ceftazidime; one was also resistant to ampicillin (Fig. 3). Among the four regrown *Pseudomonas* spp. isolates, three were resistant to trimethoprim-sulfamethoxazole, each also intermediate resistant to one other antimicrobial, either tetracycline, or piperacillin-tazobactam. Among the 10 regrown *Acinetobacter* spp. isolates, one was resistant to trimethoprim-sulfamethoxazole and one had intermediate resistance to tetracycline. All *Enterobacter* spp. and *Citrobacter* spp. isolates were pan-susceptible.

Odds of Nasal GNO Carriage by Job Category

For the 90 participants who were assessed for nasal GNOs, the adjusted odds of GNO carriage was 5.94 times (95% CI: 0.94, 37.50) and 6.29 times (95% CI: 1.43, 27.71) higher in participants from the maintenance/cleaning category

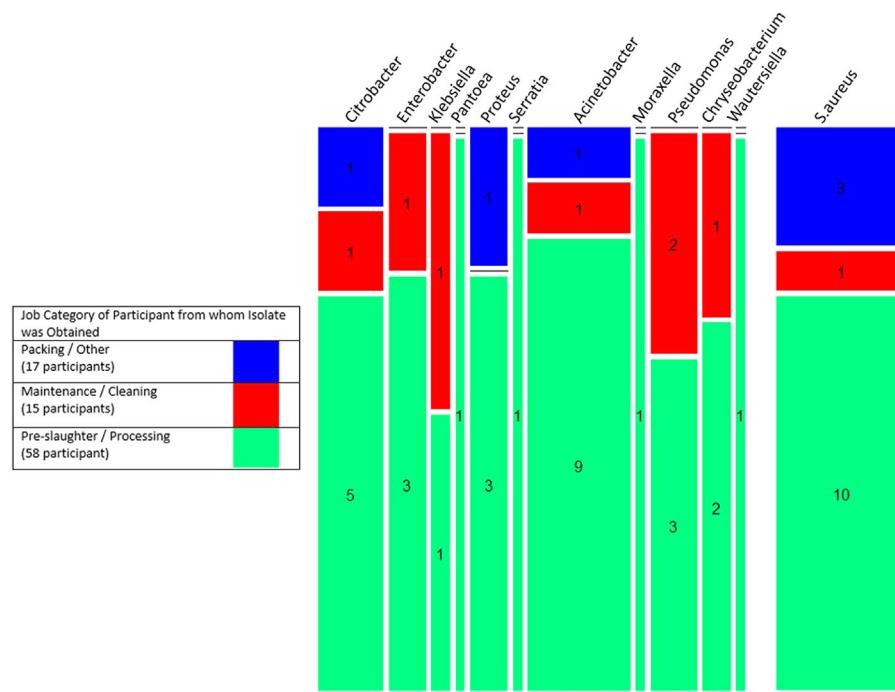


FIGURE 2. Genera of the 40 morphologically distinct GNOs and 14 *S. aureus* isolates from the 90 participants assessed for nasal GNOs, by job category. Box width reflects the percentage of isolates from each genus; box height reflects the percentage of isolates from each job category. Of these participants, 34 were positive for GNOs, 12 were positive for *S. aureus*, and 2 were positive for both.



*The antimicrobials tested were based on the CLSI standard and consultation with the Johns Hopkins Hospital Clinical Microbiology Laboratory. Some drugs were not tested for all genera due to intrinsic resistance or because they are not recommended for these genera.

FIGURE 3. GNO antimicrobial susceptibility patterns. Forty GNOs were isolated from the nares of 36 out of the 90 workers, one per individual for 32 participants and two morphologically distinct isolates per individual for four participants. We tested antimicrobial susceptibility of isolates from the most frequently detected genera: *Acinetobacter* ($n = 10$, 1 additional did not grow), *Citrobacter* ($n = 7$), *Enterobacter* ($n = 4$), *Proteus* ($n = 4$), *Pseudomonas* ($n = 4$, 1 additional did not grow). Twenty of the 29 isolates were pan-susceptible to all tested antimicrobials; 9 of the 29 isolates (31.0%) were non-susceptible to at least one antimicrobial. All *Enterobacter* and *Citrobacter* isolates were pan-susceptible to all tested antimicrobials and are not included in the figure; the results of the remaining 18 isolates are depicted below.

and from the pre-slaughter/processing category, respectively, compared to participants from the packing/other category (Table III). These odds are adjusted for gender, working in the third shift as compared to earlier shifts, and self-reported use of any antimicrobial in the last 6 months (Table III). Female gender and usage of antimicrobials in the last 6 months were associated with lower odds of nasal GNOs. Working prior to the third shift was also associated with lower odds of nasal GNOs; however, the confidence intervals for this estimate were very wide. Accounting for these covariates strengthened the unadjusted association between job category and nasal GNOs. Sensitivity analysis and alternative logistic regression models provided similar results (see SM).

DISCUSSION

To our knowledge this is the first epidemiological study in the United States to investigate nasal carriage of *S. aureus* and GNOs by poultry slaughter/processing plant workers. To our knowledge this is also the first study to examine associations between nasal carriage of GNOs and inferred job-related intensity of exposure to poultry, either via direct contact or inhalation of bioaerosols. We examined this by

comparing the association with GNO carriage for job duties entailing intensive exposure to poultry, namely those involving handling live poultry or processing carcasses and those involving maintenance or cleaning, with job duties entailing less intensive or minimal exposure to poultry, namely those involving packing poultry meat and those involving shipping or office work within the same plant. We were able to enroll 110 adult workers in two enrollment campaigns and obtain information on work and job duties, as well as other covariates by detailed questionnaire and nasal swab biosamples.

In our study, the overall prevalence of nasal carriage of *S. aureus* was 14.6%. There are no recent studies on national prevalence of nasal *S. aureus* carriage in US adults. The last population based survey, part of the National Health and Nutrition Examination Survey, reported 31.4% nasal colonization with *S. aureus* in US adults in 2001–2002 and 27.4% in 2003–2004 [Gorwitz et al., 2008]. Besides MRSA, the only non-susceptible phenotype observed in *S. aureus* in this study was non-susceptibility to erythromycin; erythromycin non-susceptibility has also been reported in *S. aureus* isolates from other slaughterhouse workers [Mulders et al., 2010, Wendlandt et al., 2013] and in isolates from retail chicken meat [Waters et al., 2011]. We found only

TABLE III. Unadjusted and Adjusted Odds Ratios Estimating the Association Between Work Duties and Covariates With Detected Nasal GNOs (Limited to Participants Tested for GNOs, N = 90)

Category	n	Unadjusted odds ratio (95% CI)	P-value	Adjusted odds ratio (95% CI)	P-value
Job category					
Packing/other	17	Referent	—	Referent	—
Maintenance/cleaning	15	4.08 (0.82, 20.38)	0.086	5.94 (0.94, 37.50)	0.058
Pre-slaughter/processing	58	3.79 (0.98, 14.63)	0.053	6.29 (1.43, 27.71)	0.015
Female gender	32	0.45 (0.18, 1.14)	0.091	0.33 (0.11, 0.93)	0.035
Works before third shift	41	0.77 (0.33, 1.80)	0.546	0.59 (0.20, 1.58)	0.272
Use of antibiotics in last 6 months	26	0.34 (0.12, 0.96)	0.041	0.39 (0.13, 1.15)	0.088

one person positive for MRSA, lower than the prevalence observed in our study of hog slaughter/processing plant workers [Castillo Neyra et al., 2014].

By comparison, there is one similar study of poultry slaughter and processing plant workers from the Netherlands. Mulders et al. [2010] reported an overall MRSA positivity rate of 5.6% in 466 workers from several processing plants. Most of the MRSA isolates they detected were ST398, a sequence type associated with livestock and poultry in several EU countries, but apparently less prevalent in the US [Waters et al., 2011; Rinsky et al., 2013; Castillo Neyra et al., 2014].

In 90 participants we also assessed nasal carriage of GNOs. While incomplete, this sampling included representatives from all of the job categories within the cohort as a whole. We found that 36 (40%) of these 90 participants were positive for nasal GNOs, of which 30 isolates were *Acinetobacter* spp. or members of the family *Enterobacteriaceae*. CDC considers monitoring these bacterial groups for development of antimicrobial resistance as a high priority [CDC, 2013]. There is limited information on nasal carriage of GNOs among persons outside of health care settings; however, sequence-based studies have reported GNOs as part of the nasal microbiome of healthy persons [Grice and Segre 2011]. Using culture-based methods, one US study of healthy military personnel reported that very few (4/101) were positive for nasal GNOs [Vento et al., 2013], while a Swedish study of 101 healthy police students reported that 14 out of their 191 nasal isolates were GNOs [Hulterström et al., 2012].

Two participants, both of whom worked in the pre-slaughter/processing category, tested positive for both *S. aureus* and GNOs. Otherwise, we observed a suggested inverse relationship between carriage of *S. aureus* and GNOs by job categories, which may be an artifact of in situ competition or in vitro isolate culture. The reported overgrowth by GNOs in our cultures may have excluded the presence or the detection of *S. aureus*.

Job category was a significant determinant of risk of nasal GNO carriage, with the participants in contact with live chickens or involved in post-slaughter carcass processing

at significantly increased risk compared to participants employed in packing, shipping, box-making, or office work. In addition, for the first time, we were able to include maintenance and cleanup workers in a study of pathogen exposures in livestock and poultry slaughter and processing. These workers are often contractual labor and thus, not included as part of the plant workforce. In our study, we recruited 21 persons in this group and screened seventeen of them for nasal GNOs. While they were at increased risk of GNO carriage as compared to participants employed in packing, shipping, box-making, or office work, these increases were of borderline statistical significance.

The observed association of prevalent nasal GNO carriage with intensity of occupational poultry contact (as approximated by job categories) provide preliminary support for the hypothesis that the detected GNOs are zoonotic in origin. Our hypothesis and results are also consistent with the results of European studies that have reported *Enterobacter*, *Citrobacter*, and *Klebsiella* as among the most frequently detected *Enterobacteriaceae* in chicken carcasses at slaughterhouses [Schwaiger et al., 2012]. Several other studies have reported GNOs as airborne microorganisms in poultry slaughter and processing plants [Whyte et al., 2001; Lues et al., 2007; Fallschissel et al., 2010; Liang et al., 2013]. *Enterobacteriaceae* including resistant *P. mirabilis* have been reported in European studies as contaminants on retail chicken [Overdevest et al., 2011; Kola et al., 2012]. Detailed molecular analyses comparing isolates from retail chicken meat with those from human rectal swabs and blood have identified chicken products as a likely part of the emergence of ESBL-producing *E. coli* in humans [Leverstein-van Hall et al., 2011; Kluytmans et al., 2012]. Similarly, a US study also suggested the origin of many drug resistant human fecal *E. coli* isolates from poultry isolates [Johnson et al., 2007].

Among other covariates associated with nasal GNO carriage, the lower prevalence of nasal GNO carriage associated with recent self-reported antimicrobial use was not surprising whereas the relatively strong association with gender was not expected. This may be partially explained by the observation that women participants more frequently

reported consistent use of face masks than men. There may be additional differences in actual work performed, which were not captured in our study. We also found an association of nasal GNO carriage with shift (before third versus third), the direction of which was unexpected since plant cleaning occurs during the second shift and the plant is presumably cleaner during the third shift. However, there is considerable uncertainty in the direction of this estimate. It is also possible that additional unmeasured differences between the day (first and second) and night (third) shifts influenced GNO carriage.

We recognize the limitations of our study. As it is a cross-sectional study, we lack temporal information on the workers' nasal colonization status prior to enrollment or independent of their employment at the plant. Similarly, information on persistent nasal colonization of the detected pathogens or on subsequent incidences of infections for this workforce is lacking. Participants were enrolled by convenience and thus not fully representative of the workforce in the plant as a whole. Moreover, our sample size was limited, with fewer participants in the category of maintenance/cleaning and packing/other. Our ability to enroll maintenance/cleaning workers was not anticipated, as this workforce is not usually unionized in US plants. Also, the enrolled cohort was not all screened for GNOs. This further decreased the size of the maintenance/cleaning and packing/other categories, as these two categories had relatively lower proportions of participants screened for GNOs. A larger sample size, particularly for the packing/other and maintenance/cleaning categories, may yield more conclusive results. Categorization of occupational exposures was based on department assignment and open-ended job descriptions, which varied considerably among participants. Additionally, several participants reported doing multiple jobs. Thus, the assigned job categories, particularly the collapsed categories, do not reflect the potential heterogeneity of day-to-day job duties for some workers. Due to these limitations of one-sentence self-reported job summaries, overlap of actual day-to-day duties across the assigned job categories is likely, although its extent cannot be quantified without more information. However, the mis-categorizations from this overlap are highly unlikely to be related to the measured or actual nasal GNO status. The expected result of this type of mixing within the job categories (i.e., non-differential, independent measurement error), with each category containing a mixture of individuals with relatively higher and lower intensities of poultry contact, would be attenuated estimates of the differences in nasal GNO carriage among the job categories. Future studies could be improved by using more targeted questions about job duties. Also, our findings on GNO exposure and overall prevalence are limited to analyses of nasal swabs, since our study was not planned to conduct more extensive biosampling including feces and skin. In contrast to nasal carriage of *S. aureus*, the clinical interpretation of nasal GNOs is less clear; these results are

appropriately interpreted as a starting point for further investigation, particularly of the detected GNOs on the CDC priority list for monitoring antimicrobial resistance.

This study is the first report on these exposures in US poultry slaughter and processing plants. The conditions in this plant may not be representative of the industry as a whole, which indicates the need for further research. We examined GNO exposures on a relative basis by work area and job duty within this occupational cohort, but our evaluation of exposure is not based on actual sampling of the workplace (which is not possible in the US). Still, other studies examining actual workplaces support our exposure categorization of the workforce in this article [Whyte et al., 2001; Lues et al., 2007; Liang et al., 2013]. The inclusion of a non-worker referent population would improve estimates of the risk of nasal GNO carriage due to exposures from the poultry slaughterhouse environment. It is important to note that this study did not evaluate prevalence of infection or disease.

Finally, while the associations observed between job duties and prevalence of nasal carriage of certain pathogens is consistent with the workplace as a source of exposure, this study cannot identify the source of exposure as zoonotic without further information on the pathogens present in poultry and poultry products within the workplace. This is a general limitation of most studies conducted on workers, especially in the US, where access to slaughter and processing operations is difficult and no reports by either government or industry are available on this topic.

CONCLUSIONS

This study suggests that poultry slaughter/processing plant workers who are in frequent contact with live poultry and/or carcasses as well as cleanup and maintenance workers may be at increased risk of exposure to GNOs, a sizeable proportion of which are non-susceptible to antimicrobials, as compared to workers in packing, shipping, box-making, and office jobs at the same plant. Our study is consistent with previous work by us and others on other zoonotic pathogens in this workforce [Castillo Neyra et al., 2012; CDC NIOSH, 2014]. Finally these findings have broader public health implications, including evaluating job-related exposures to pathogens as an occupational risk for workers in this industry, as well as the potential for transmission from workers to their communities [Castillo Neyra et al., 2012]. Our data add to concerns about poultry as a potential source of pathogenic and drug resistant GNOs in terms of food borne exposures.

AUTHORS' CONTRIBUTIONS

Y.Y., K.L., and E.K.S. conceived the study. C.R., E.K.S., T.H., and Y.Y. acquired the data; K.L., Y.Y., and E.K.S. analyzed the data, and prepared the manuscript; all authors

contributed to manuscript editing. Y.Y. and K.L. contributed equally to the research and writing of this paper and are co-first authors.

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ETHICS REVIEW AND APPROVAL

This study was reviewed and approved by the Johns Hopkins School of Public Health Institutional Review Board. Participants provided verbal consent to a form that was read to them prior to participation. Written informed consent was not taken to ensure participants' confidentiality.

DISCLOSURE (AUTHORS)

The authors declare they have no actual or potential competing financial interests. No funds or other support were received from the UFCW for this research.

DISCLOSURE BY AJIM EDITOR OF RECORD

Paul Landsbergis declares that he has no competing or conflicts of interest in the review and publication decision regarding this article.

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