Baseline Data on Enumerated Tracheal Bacterial Flora and Drug Susceptibility in Chicken Reared Under Different Systems

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ABSTRACT

The aim of this study is to establish baseline data on enumeration and drug resistance of selected microbiota in the upper respiratory system of healthy layer and broiler chickens under free range and intensive environmental systems.

A total of 232 tracheal swabs from individual chickens were cultured for enumeration of 5 selected bacterial groups. Results showed that the count of total bacteria, *Staphylococcus aureus*, and the psychrophilic bacteria in the upper respiratory system were not significantly different ($P > 0.05$) in the various types of birds reared under the 2 systems, whereas the tracheal coliform count was significantly higher in broilers compared with layers regardless of the system type ($P < 0.05$). Conversely, the tracheal bacterial resistance to chloramphenicol was significantly higher in broilers compared with layers ($P < 0.05$); resistance to ciprofloxacin was similarly high in all types of chickens and systems ($P > 0.05$). In addition, resistance to gentamycin was higher in broilers compared with layers and in free-range birds compared with birds in intensive systems ($P < 0.05$).

INTRODUCTION

During the last few decades, intensive animal production had acquired a major importance in the market in order to satisfy the needs of a growing consuming population.[1] Although very effective in some ways, however, intensive production is the cause of many health and environmental problems,

and it was only a few years ago that people started to recognize the potential hazards of such practices and the urge to initiate new healthier products.[2–4] Free-range production is still a very small proportion of the total market, but it is increasing in size day after day due to its greater potential in satisfying the animal welfare objectives in comparison with industrial systems.[2] In the standards of free-range production, the farm requisites must take into consideration the importance of environmental implications, such as clean air and floors and the existence of outdoor housings in addition to the physiological and behavioral needs of animals.[2,5] Furthermore, antimicrobials should be used in a very limited amount, not for growth promotion but rather for respectively curing and preventing against spreading diseases, something that is not the case in most poultry intensive systems existing around the world.[2]

This study aims at comparing the effect of environmental conditions on the upper respiratory microbiota density of healthy broiler and layer chickens as it is in direct contact with the air that chickens breath and may reflect the general environmental conditions. In addition, tracheal bacterial drug susceptibility to 3 antimicrobials (gentamycin, chloramphenicol, and ciprofloxacin) was also studied to observe the extent of tracheal bacterial drug resistance in both types of chicken under the 2 environmental systems. These specific antimicrobials were selected because the World Health Organization (WHO) has recommended their prohibition in all food-producing animals in order to help reducing human bacterial resistance to these important drugs.[6,7] To our knowledge, this is the first attempt in establishing baseline data on tracheal bacterial density in healthy chickens under different environmental systems. The establishment of a baseline on healthy chicken tracheal microbiota density will help in future investigations to understand the dynamics of the shifts towards damaging higher tracheal bacterial densities and drug-resistance emergence, both during developmental stages of different respiratory diseases and post-chemotherapy.

MATERIALS AND METHODS

Experimental Design

A total of 232 chickens were studied, 128 of which were randomly selected from flocks raised in an industrial system and 104 from a free-range system. Seven free-range farms were included in the study, 4 of which consisted of layer chickens and 3 of broiler chickens; additionally, 6 industrial farms were randomly chosen, 3 of which contained broiler chickens and 3 of layer chickens. The age of layers and broilers ranged between 10 to 15 months and 1.5 to 3 months, respectively. The average size of flocks in farms of free-range layers, intensive layers, free-range broilers, and intensive broilers were 200, 7000, 500, and 7000, respectively. The number of individual tracheal swabs from each flock of the mentioned types of farms ranged from 10–20, 20–22, 20–30, and 20–22, respectively.

Regarding environment, chickens growing in the free-range system had access to an outdoor backyard with fresh air, sunlight, and natural food; their average density was 1.2 chickens per m^2. Chickens growing in the industrial system were allowed a much smaller area, namely 11 and 6 chickens per m^2 for broilers and layers, respectively.

Sampling and Transportation of Swabs

Sterile cotton swabs were used to sample bacteria from individual trachea of birds. Each swab was shaken gently in 2.5 mL of sterile phosphate buffer saline (PBS) to suspend the sampled bacteria. The suspended bacteria were transported in an ice chest and cultured for enumeration and drug susceptibility testing within 1 hour following sampling.

Enumeration of Bacteria From the Upper Respiratory System

The choice of the selected groups of bacteria was based on their capacity to exist in the natural environment of poultry and/or their potential threat in respiratory diseases.[8–10]

Table 1: Mean of Averages* of Tracheal Bacterial Group Counts From Different Chicken Types Reared Under Different Systems.†

Enumerated Tracheal Bacterial Groups (CFU/Swab)	Layer Systems		Broiler Systems	
	Free Range	Industrial	Free Range	Industrial
Total count	1.08×10^{6}	1.11×10^{6}	4.83×10^{4}	7.22×10^{4}
Coliform count	1.88×10^{3a}	4.50×10^{2a}	1.08×10^{4b}	7.22×10^{3b}
Staphylococcus aureus count	2.13×10^{4}	6.60×10^{3}	4.17×10^{4}	3.33×10^{4}
Psychrophilic count	8.10×10^{3}	1.18×10^{3}	1.58×10^{5}	6.31×10^{5}

*Averages of tracheal bacterial counts on each farm were obtained, and then the mean of averages of the farms of same chicken type and system were compared statistically to other chicken types and systems.

†Number of free range layer farms is equal to 4 with 52 total tracheal samples, while the number of industrial layer farms is equal to 3 with 64 total tracheal samples; the number of free range broiler farms is equal to 3 with 52 total tracheal samples and the number of industrial broiler farms is equal to 3 with 64 total tracheal samples.

[a,b]Mean of averages within a row that are followed by different superscripts are significantly different ($P < 0.05$).

Five groups of bacteria were enumerated, namely: total bacteria, coliforms, psychrophilic bacteria, *Staphylococcus aureus*, and *Clostridium perfringens*. The enumeration was done according to a previously described method.[11,12] Briefly, the bacterial suspension was subjected to serial dilutions with a dilution factor of 1/10. A volume of 0.1 mL of each dilution was spread over nutrient agar (NA) for total and psychrophilic bacteria, MacConkey agar for coliforms, and mannitol salt agar (MSA) for *S. aureus* and trypcase sulfite neomycin (TSN) for *C. perfringens*. Plates were incubated upside down at 37˚ C for 24 hours, except for the psychrophilic NA plates, which were placed in a refrigerator at a temperature between 4˚ C and 6˚ C for a period of 10 days. A volume of 0.5 mL of each original tracheal bacterial suspension was added into an empty Petri dish, then overlaid with 20 mL of the TSN medium that was set at 45˚ C in a water bath. The inoculum and the TSN agar were mixed by "8 number" movements and kept at room temperature for about 10 minutes to solidify. The TSN plates were incubated at 37˚ C for a period of 48 hours while kept in an anaerobic jar with palladium catalyst and $CO_2 + H_2$ liberating envelops. Colonies were counted and the colony forming units (CFU) were reported per tracheal swab.

Antimicrobial Susceptibility

The main PBS tracheal bacterial suspensions were used for the purpose of studying bacterial drug resistance by the Kirby Bauer method.[13] Briefly, a volume of 0.1 mL of each main suspension was spread by L-glass rod over individual Mueller Hinton agar (MHA) plates. Three antimicrobial agents present on paper discs, namely, gentamicin (10 μg), chloramphenicol (30 μg), and ciprofloxacin (5 μg), were laid over each spread culture. The plates were then incubated upside down at 37˚ C for a period of 24 hours. The diameter of the inhibition zone was measured in mm, and the susceptibility was interpreted by the help of a chart provided by the disc manufacturer (BBL, Baltimore, Maryland).

Statistical Analysis

Statistical analyses were performed using the complete randomized design, 2×2×2 factorial analysis in order to compare mean bacterial group counts in broiler and layer chicken reared under different environments (free range and industrial). The same method was applied for comparing resistance of tracheal bacteria to each drug. The SPSS version 12 (SPSS. INC. Headquarters Chicago, Illinois, 2004) was used for this statistical computing. Means were compared using Duncan multiple range test.

RESULTS AND DISCUSSION

The total bacterial count, *S. aureus*, and the psychrophilic bacteria enumerated in the tracheal swabs of different types of birds under different systems were not signifi-

Table 2: Comparison of the Mean Percentage* Tracheal Bacterial Susceptibility to Drugs in Layers and Broilers Under Different Systems.

Poultry Type	Management System	Number of Farms	Number of Tracheal Swabs	Mean Percent Tracheal Bacterial Drug-Susceptibility								
				Resistant			Intermediate			Sensitive		
				GENT	CHLOR	CIPRO	GENT	CHLOR	CIPRO	GENT	CHLOR	CIPRO
Layers	Free range	4	52	56.01[a]	63.52[a]	95.23	7.28	22.61	1.25	36.71	13.87	3.52
	Industrial	3	64	31.40[b]	76.90[a]	92.44	23.90	10.91	3.03	44.70	12.19	4.53
Broilers	Free range	3	52	90.90[c]	97.74[b]	95.45	2.27	0.00	4.55	6.83	2.26	0.00
	Industrial	3	64	84.42[d]	96.85[b]	81.54	7.72	0.00	7.86	7.86	3.15	10.60

GENT = gentamycin; CHLOR = chloramphenicol; CIPRO = ciprofloxavin.

*The percentage susceptibility of tracheal cultures from each sampled farm was recorded, and the mean of same type of poultry farms under a specific system is shown in the table.

[a,b,c,d]Means in a column followed by different superscripts are significantly different ($P < 0.05$).

cantly different ($P > 0.05$) (Table 1). This may indicate that the density of the receptors in the trachea is most likely similar in the different kinds of birds; thus, the density of colonization by the total bacteria is identical irrespective of the environmental system. Another possible reason for this similarity in the enumerated total bacteria could be the fact that the free-range birds are confined just after sunset to their indoor barn for a night period of about 12 hours, a long period of exposure to their dry litter that works as a source of dust in their closed environment; the dust particles are known to carry microorganisms of various kinds.[14]

There was a highly significant difference of enumerated tracheal coliforms between layers and broilers regardless of the system type ($P < 0.05$). Layers always had less average coliform counts than broilers (Table 1). This could be due to the difference in the feed formulations between layers and broilers,[15] a factor that could affect the level of coliforms shed in the litter, leading to differences in the density of coliforms in the air and later in the colonized trachea. It is always observed that broiler litter is wetter than the layer litter, causing more fermentation and ammonia generation.[16] A high ammonia level in the air of broiler houses could cause more deciliation, which leads to the loss of the first line of defense against colonization by coliforms. Actually, coliforms are rated as number one in secondary infections of broilers around the world.[17] In addition, the sampled free-range and intensive broiler farms follow continuous lighting programs (24/24 hours), while in the sampled free-range and intensive layer farms, the average light hours per day is 15. This makes a difference in feed intake and temperature in the barn due to difference in the light bulbs that are kept on for different times in both types of birds, most likely leading to higher levels of coliforms in the broiler barns and consequently in the bird's trachea.

Regarding *C. perfringens* bacteria, they were not found in any of the tracheal cul-

tures collected from chicken reared in free-range or industrial locations. This is most likely due to the absence of receptors in the trachea to *C. perfringens* organisms. It is documented that this organism is an enteric pathogen in chicken and not a respiratory etiologic agent.[18]

The bacterial resistance to the chloramphenicol antimicrobial in the poultry trachea was significantly higher in broilers compared with layers (Table 2), regardless of the environmental system ($P < 0.05$). On one hand, this is an expected result, since many farmers in the developing world still use chloramphenicol on a wide scale in broilers to help suppress secondary infections by coliforms following a whole spectrum of primary causative etiologic agents.[19,20] On the other hand, layers are bred for efficient production of eggs and for resistance to many economical diseases, making them less susceptible to bacterial invasion, thus less in need for chemotherapy. There was a similar high resistance of tracheal bacteria to ciprofloxacin in both types of chicken and under different environmental systems ($P > 0.05$) (Table 2). This is most likely due to its application in both layers and broilers as a preventive and treatment medication. In addition, its wide spectrum of activity against both Gram-positive and Gram-negative bacteria leads to emergence of resistant strains, where the percentage resistance in this study in the various bird types reared under different systems ranged between 81.54% and 95.45%, a range that is considered very high. Other countries reported lower resistance to members of the fluoroquinolones family, such as 23% resistance in Morocco,[21] 30% resistance in Trinidad,[22] 11.3% resistance in Taiwan,[23] and 40% resistance in Georgia, USA.[8] The resistance of tracheal flora in this study to gentamycin ranged between 31.4% and 90.90% (Table 2); the tracheal bacteria from broilers were significantly higher in their resistance in comparison to layers ($P < 0.05$). The same reason given before applies here, where broilers farmers are faced with recurrent diseases outbreaks usually due to a lack of National Poultry Improvement Plan in developing countries, forcing them to use all kinds of antimicrobials, including the ones that are prohibited by the WHO and the FDA, such as chloramphenicol and gentamycin. Other countries' reports are still showing less bacterial resistance in poultry to gentamycin, including the 7% resistance reported from Morocco[21] and the 50% resistance reported from Trinidad.[22] On the contrary, other neighboring Middle Eastern countries, namely, Saudi Arabia, showed a high resistance to gentamycin (89.7% resistance). The data indicate more tracheal bacterial resistance ($P < 0.05$) to gentamycin in free-range birds compared to industrial birds, regardless of the bird type. This is most probably due to obtaining the birds for free range rearing from industrially grown parents that were treated continuously with antimicrobials, thus producing offspring in hatcheries that are carriers of resistant bacteria transmitted from their parents through infected ovaries and oviducts or through egg shell pores.[24] In addition, at the present time, and due to a lack of governmental policing of free-range farming, this could lead to unreported use of antimicrobials in their vicinity. The results obtained in this study demonstrate that it is crucial to implement a National Poultry Improvement Plan in each poultry-producing country that would improve farm management and lead to better monitoring of antimicrobial use in both free-range and industrial environmental systems, thereby improving the quality of poultry meat by reducing its contamination with drug resistant bacterial microbiota, and thus protecting the end user, the human consumers.

CONCLUSION AND RECOMMENDATIONS

According to the results obtained in this study, it is crucial to implement a National Poultry Improvement Plan to monitor antimicrobial use in both free-range and

industrial management systems and to attempt to suppress the density of the upper respiratory system bacteria to a level that is less than the infective dose. This will decrease the chance of bacterial disease outbreaks and, by that, lessen the need for drugs and the possible contamination of consumed meat.

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