Bacterial Quality Assessment of Drinking Water for Layer Chicken Managed Under Battery Cage and Deep Litter Systems from Sokoto Metropolis, Nigeria

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ABSTRACT: This study assessed the bacterial quality of drinking water for layer chicken managed under battery cage (BC) and deep litter (DL) systems in Sokoto Metropolis. A total of 18 samples were collected from the two systems. Serial dilution, spread plate inoculation, colony count, subculturing, gram staining and biochemical characterization were carried out according to standard methods. The mean count concentrations in BC (1.4×10⁶, 7.2×10⁶ and 3.4×10⁶) were relatively higher than those recorded in DL (1.57×10⁶, 4.52×10⁶, and 1.2×10⁶). The mean count (CFU/ml) for BC was 72.1111 and that of DL was 207.4444. The bacteria determined in BC were: Bacillus species, Micrococcus varians, Corynebacterium xerosis and Lactobacillus fermenti; whereas, those determined in DL were Micrococcus varians, Lactobacillus fermenti, E. coli, and Corynebacterium xerosis; thus E. coli was only recorded in DC, but the rest were found in both BC and DL. In BC, the most frequent was Corynebacterium xerosis, then Micrococcus varians, and lastly Bacillus species and Lactobacillus fermenti; whereas, in DL Corynebacterium xerosis was also most frequent, then Micrococcus varians then the rest. Thus, C. xerosis was the most overall prevalent, then Micrococcus varians, then the rests. This work depicted that water used in the BC and DL systems surveyed contains a higher and diverse concentration of bacteria. This portend of contamination and unsanitary outcome is capable of harming the health, production, and ultimately the public health. More water treatment innovative methods should be use, regular and proper cleaning of farm and drinkers are needed and farmers need to be educated.

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Poultry is a source of food that has been accepted worldwide through the ages. The consumption of poultry products is increasing every year and consumers want a safe product, thus it is pertinent that the poultry producers achieved this goal. Often, poultry products are involved in human foodborne poisoning/diseases posing a considerable cost and threat to public health (International Consultative Group on Food Irradiation, 1999; Ventura daSilva, 2013; Sule and Ilori, 2017). Increase in contacts of poultry with microbes lead to increased contact rates with humans and open new avenues for introduction, proliferation, and transmission of pathogens; and ultimately more threats to public health (The PEW Charitable Trusts, 2016). Two major poultry systems in Nigeria and Sokoto in particular are the Deep litter (DL), where birds are reared in restricted houses; and Battery cage (BC), where birds are reared in cages(Adam, 2017). Therein, quality water is essential for proper production and safety of poultry health and consequently public health (Folorunso et al., 2004; Abbas et al., 2008). Water is used in electrolyte replacement therapy, treatment with drugs, and cleaning among others. But the quality of drinking water in poultry can be jeopardized as a result of diverse things. Parable, the source (well or pipe), poor cleaning and maintenance of drinkers, regurgitated feed by the birds, chicken feed, chicken conduct, rearing sites, faeces, antimicrobials or drugs, and knowledge of rearers (Folorunso et al., 2014; Oviasogie et al., 2016). Consequently, the objective of this paper was to determine the bacterial quality of drinking water for layer chicken managed under deep litter (DL) and battery cage (BL) systems in Sokoto, Nigeria.

MATERIALS AND METHODS

Sample collection: A total of 18 samples were collected randomly from 3 farms of DL and BC in Sokoto.

Sterilization, and Preparation of media: All glass wares were sterilized using standard methods outlined in Oviasogie et al.,(2016). Nutrient agar was prepared according to the standard procedure outlined by Microbiology Society (2016). Simmons Citrate agar,
Triple Sugar Iron agar and urea agar were prepared based on methods reported in HiMedia Laboratories (2019), HiMedia Laboratories (2015), and Downes (2001) respectively. Indole agar was prepared according to protocols stated by MacWilliams (2009).

**Microbial Analysis:** Serial dilution, inoculation (spread plate method), bacterial counting, gram staining, and subculturing were performed based on standard methods outlined by Folorunso et al., (2014), Cheesbrough (2009), and Microbiology Society (2016). Biochemical characterization of microbes was carried out according to Cheesbrough (2009).

**Statistical Analyses:** Data was analyzed using descriptive statistics (percentage, range, means, and standard deviation). T-test was carried out to compare the 2 housing systems using Statistical Analysis Software (SAS, 2002).

**RESULTS AND DISCUSSIONS**

Table 1 has shown the bacterial count between weeks 1-3 from samples collected from water troughs in BC and DL systems. The mean count concentrations in BC (1.4×10^6, 7.2×10^6, and 3.4×10^6) were relatively higher than those recorded in DL (1.57×10^7, 4.52×10^7, and 1.2×10^7). This may be why the body weight of chicken from DL systems was higher as echoed by Adam (2017). The results contradict reports from Folorunso et al., (2014). All the values (concentrations) recorded were high, similar to a Southeastern study reported by Folorunso et al., (2014). This finding points to a contamination point(s)/sources that endanger the quality of drinking water in the study birds and can ultimately harm production and public health (ICG, 1999; Abbas et al., 2008; Food Standards Australia New Zealand, 2008). Microbes in water or other contacts with the bird enters eggs and kill them or make them unhealthy to consumers (Abbas et al., 2008).

| Housing system | Farm | Weeks | No. of colonies | Mean count (cfu/ml) | Standard (cfu/ml) |
|----------------|------|-------|-----------------|---------------------|------------------|
| Battery cage   | Farm 1 | Week 1 | 15              | 1.5×10^6           |                  |
|                | Week 2 | 12    | 1.4×10^6        | 1.2×10^6           |                  |
|                | Week 3 | 15    | 1.5×10^6        | 1.5×10^6           |                  |
|                | Farm 2 | Week 1 | 124             | 1.24×10^7          |                  |
|                | Week 2 | 61    | 7.2×10^6        | 6.1×10^6           |                  |
|                | Week 3 | 320   | 3.2×10^7        |                    |                  |
|                | Farm 3 | Week 1 | 96              | 9.6×10^6           |                  |
|                | Week 2 | 3     | 3.4×10^4        | 0.3×10^5           |                  |
|                | Week 3 | 3     | 3.3×10^3        | 0.3×10^3           |                  |
| Deep litter    | Farm 1 | Week 1 | 51              | 5.1×10^6           |                  |
|                | Week 2 | 194   | 1.57×10^7       | 1.94×10^7          |                  |
|                | Week 3 | 228   | 2.28×10^7       |                    |                  |
|                | Farm 2 | Week 1 | 640             | 6.40×10^7          |                  |
|                | Week 2 | 111   | 4.52×10^7       | 1.11×10^7          |                  |
|                | Week 3 | 606   | 6.05×10^7       |                    |                  |
|                | Farm 3 | Week 1 | 8               | 0.8×10^7           |                  |
|                | Week 2 | 22    | 1.2×10^6        | 2.2×10^6           |                  |
|                | Week 3 | 7     | 0.7×10^5        |                    |                  |

In table 2, the bacterial species associated with BC and DL systems of this study were shown. The mean count (CFU/ml) for BC was 72.11111 and that of DL was 207.4444. The bacteria determined in BC envisaged: *Bacillus* species, *Micrococcus* varians, *Corynebacterium xerosis* and *Lactobacillus fermenti*; whereas, those determined in DL are *Micrococcus* varians, *Lactobacillus fermenti*, *E. coli*, and *Corynebacterium xerosis*; thus *E. coli* was only recorded in DC, but the rest were found in both BC and DL. Folorunso et al., (2014) observed *E. coli*, *Bacillus* species, and *Corynebacterium species*.

Table 3 depicted the frequency of occurrence of the bacterial species in BC and DL systems of this study. In BC, the most frequent was *Corynebacterium xerosis*, then *Micrococcus* varians, and lastly *Bacillus* species and *Lactobacillus fermenti*. Whereas, in DL *Corynebacterium xerosis* was also most frequent, then *Micrococcus* varians, then the rests. Thus, *Corynebacterium xerosis* was the most overall prevalent, then *M. varians*, then the rests. Sule and Ilori (2017) determined *Micrococcus* species (more particularly *M. varians*) from poultry feed in Ilorin, Nigeria. *Lactobacillus* bacteria are nonpathogenic microbes that naturally inhabit the mucous of humans and animals (including chickens) providing a protective barrier in the gut.
Table 2: Bacterial load (CFU/ml) of species associated with drinking water under BC and DL in Sokoto metropolis

| Housing system | Farm | Weeks | Range count (cfu/ml) | Bacteria species                  |
|----------------|------|-------|---------------------|-----------------------------------|
|                | Farm 1 | Week 1 | $1.5 \times 10^9$  | Bacillus species                  |
|                |       | Week 2 | $1.5 \times 10^9$  | Micrococcus varians               |
| Battery cage   |       | Week 3 | $1.2 \times 10^9$  | Corynebacterium xerosis           |
|                | Farm 2 | Week 1 | $3.2 \times 10^7$  | Lactobacillus fermenti            |
|                |       | Week 2 | $1.24 \times 10^7$ | Corynebacterium xerosis           |
|                |       | Week 3 | $6.1 \times 10^9$  | Micrococcus varians               |
|                | Farm 3 | Week 1 | $9.6 \times 10^9$  | Corynebacterium xerosis           |
|                |       | Week 2 | $0.3 \times 10^9$  | Micrococcus varians               |
|                |       | Week 3 | $0.3 \times 10^9$  | Corynebacterium xerosis           |
|                | Farm 1 | Week 1 | $2.28 \times 10^7$ | Battery cage                      |
|                |       | Week 2 | $1.97 \times 10^7$ | Micrococcus varians               |
|                |       | Week 3 | $5.1 \times 10^7$  | Corynebacterium xerosis           |
|                | Farm 2 | Week 1 | $6.4 \times 10^7$  | Micrococcus varians               |
|                |       | Week 2 | $6.06 \times 10^7$ | Micrococcus varians               |
|                |       | Week 3 | $1.11 \times 10^7$ | Lactobacillus fermenti            |
|                | Farm 3 | Week 1 | $2.2 \times 10^6$  | Micrococcus varians               |
|                |       | Week 2 | $0.8 \times 10^6$  | Bacillus species                  |
|                |       | Week 3 | $0.7 \times 10^6$  | Corynebacterium xerosis           |
|                | Farm 1 | Week 1 | $2.2 \times 10^6$  | Micrococcus varians               |
|                |       | Week 2 | $0.8 \times 10^6$  | Bacillus species                  |
|                |       | Week 3 | $0.6 \times 10^6$  | Corynebacterium xerosis           |
|                | Farm 2 | Week 1 | $6.4 \times 10^6$  | Battery cage                      |
|                |       | Week 2 | $6.06 \times 10^6$ | Micrococcus varians               |
|                |       | Week 3 | $1.11 \times 10^6$ | Lactobacillus fermenti            |
|                | Farm 3 | Week 1 | $2.2 \times 10^6$  | Micrococcus varians               |
|                |       | Week 2 | $0.8 \times 10^6$  | Bacillus species                  |
|                |       | Week 3 | $0.7 \times 10^6$  | Corynebacterium xerosis           |

It eliminate unfavourable microflora through diverse mechanisms such as production of organic acids, hydrogen peroxide, etc as inhibitors; blocking adhesion sites of epithelial, competition for nutrients and triggering of immunity. Thus, it is administered as probiotic in chicken's feed. Therefore, it is not uncommon to determine it in drinking water in this study (Gusils et al., 1998; Dec et al., 2018). Some *E. coli* (parable, Avian Pathogenic *E. coli*, APEC) causes colibacillosis, a major bacterial disease of poultry worldwide and it is communicable to humans. Some *E.coli* can traverse to all organs (in birds) and cause fatal disease (Ibrahim et al., 2019). *E. coli* commonly form biofilm, an assembly of microbial cells that is surrounded by a matrix of extrapleromic substance released by the cells. It can stay alone or attract other microbes. Growing in biofilms confers intrinsically more resistance to antimicrobials of about 1,000 fold; therefore need more drugs (Ugwoke, et al., 2019). *E. coli* reduces weight of poultry (ElSaidy et al., 2015). *E. coli* in poultry water was determined by past studies such as Ibitoye et al., (2013) from Sokoto. Aliyu et al., (2012) determined it in diverse poultry feeds in Sokoto. *Bacillus* species are responsible for food poisoning in many cases (Cunningham, 1982). Aliyu et al., (2013) observed them in poultry feed in Sokoto. *Corynebacterium xerosis* is part of the genus of *Corynebacterium species*, which have been reported in chicken and have been suspected for causing food poisoning and spoilage and it remained as an indicator of unsanitary food handling (Alibi et al., 2016).
This work illustrated that water used in the BC and DL systems surveyed contains a higher and diverse concentration of bacteria namely, *Bacillus species*, *Corynebacterium xerosis*, *Corynebacterium xerosi*, *Micrococcus varians*, *E.coli*, and *Bacillus species*. This is a portend of contamination and unsanitary outcome which is capable of harming the health, production, and ultimately the public health. Ideally, points of contamination in water are diverse. The source of water (e.g. well, pipeborne), improper cleaning and maintenance of drinkers or rearing place, feeds, drugs, faeces, farmers awareness or education are among the factors that triggers water contamination. Therefore, farmers should be made aware, and innovative systems of water treatment should be applied, proper cleaning of drinkers and cages or farms are mostly needed in order to safeguard poultry production and public health (Cunningham,1982; Amaral, 2005; Uwaezuoke and Ogbulie, 2008; Aliyu et al., 2013; Dhaka et al., 2013; Ibitoye et al., 2013; Elsaidy et al., 2015; Sarkingobir and Sarkingobir, 2017; Sarkingobir et al., 2019).

**Conclusion:** The microbes determined in this study were in high concentration, therefore the affected waters were contaminated.

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**Table 4:** Biochemical characterization of bacterial species identified from farms at Sokoto metropolis

| Sample type | Gram reaction | Catalase | SH | Lactose | Glucose | Sucrose | Citrate | Motility | Indole | Urease | MR | VP | H2S | Gas | Spore | Confirmation |
|-------------|---------------|----------|----|---------|---------|---------|---------|----------|--------|--------|----|----|-----|-----|--------|--------------|
| BC1         | + rod         | +        | -  | -       | +       | -       | LR      | -        | +      | -      | +  | -  | -   | -   | -     | Bacillus species |
| BC1         | + cocci       | +        | NA | +       | -       | -       | +       | +        | +      | SR     | -  | -  | -   | -   | -     | Micrococcus xerosis |
| BC1         | - rod         | +        | -  | +       | -       | -       | +       | +        | +      | +      | SR | -  | -   | -   | -     | Corynebacterium xerosis |
| DL1         | + rod         | +        | -  | +       | -       | +       | SR      | +        | +      | +      | SR | -  | -   | -   | -     | Corynebacterium xerosis |
| DL1         | + cocci       | +        | NA | +       | +       | +       | -       | +        | -      | -      | -  | +  | -   | -   | -     | Micrococcus varians |
| BC2         | + rod         | +        | -  | +       | -       | +       | +       | -        | +      | SR     | -  | -  | -   | +   | +     | Lactobacillus fermenti |
| DL1         | + rod         | +        | -  | +       | +       | +       | +       | +        | +      | +      | SR | -  | -   | -   | -     | Corynebacterium xerosis |
| DL2         | + rod         | +        | -  | +       | +       | -       | +       | -        | +      | -      | -  | +  | -   | -   | +     | Lactobacillus fermenti |
| DL1         | + cocci       | +        | NA | +       | +       | +       | -       | +        | -      | -      | -  | +  | -   | -   | -     | Micrococcus varians |
| BC3         | + rod         | +        | -  | -       | +       | -       | +       | -        | +      | -      | -  | +  | -   | -   | +     | Corynebacterium xerosis |
| BC3         | + cocci       | +        | NA | +       | +       | -       | +       | -        | +      | -      | -  | +  | -   | -   | -     | Micrococcus varians |
| DL3         | + rod         | +        | -  | +       | +       | +       | -       | +        | -      | -      | -  | +  | -   | -   | -     | Corynebacterium xerosis |
| DL3         | + cocci       | +        | NA | +       | +       | LR      | +        | +        | -      | +      | -  | +  | -   | -   | +     | E.coli |

**KEY:** MR=methyl red VP=vokes-proskeur NA = not applicable SR= slow reaction LR=low reaction BC=battery cage DL= deep litter
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