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Received: 22 Feb 2025 - **Accepted:** 02 Oct 2025 - **Published:** 03 Nov 2025

Keywords: Prevalence, commensal bacteria, opportunistic pathogens, transmission risk, One Health

Funding: This work received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Cite this article: Ann Munene et al. Prevalence of *Escherichia coli* and *Enterococcus* in poultry farms in Kiambu County, Kenya: a One Health approach. PAMJ-One Health. 2025;18(14). 10.11604/pamj-oh.2025.18.14.46978

Available online at: https://www.one-health.panafrican-med-journal.com/content/article/18/14/full

Prevalence of *Escherichia coli*and *Enterococcus*in poultry farms in Kiambu County, Kenya: a One Health approach

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Abstract

Introduction: *the commensal bacteria, Escherichia coli and Enterococcus spp. are abundant in the gastrointestinal tract of humans and animals. However, these two bacteria could also turn out to be opportunistic pathogens, as well as carriers of antimicrobial resistance genes, leading to a public health hazard in the human, animal, and environmental domains.* ***Methods:*** *this cross-sectional study investigated the prevalence of bacteria in chicken and their link to humans and the environment. Various microbiological isolation and characterization techniques were employed to identify Escherichia coliand Enterococcusspp. across all the variables investigated under the one health approach.* ***Results:*** *heavy contamination (>300 colony-forming units) with the two study bacteria was recorded on all the chicken handlers' hands. A prevalence of above 80% of Enterococcus spp. was observed across all the variables investigated. The same prevalence was noted for E. coli, with an exceptional prevalence of 10.8% in chicken handlers' hands. E. coli and Enterococcus were highly prevalent in chicken and environmental samples, with greater than 95% positivity, while only 9.8% of human pathogens were positive for both pathogens. Prevalence of E. coli (χ^2= 331.22, p < 0.001) and Enterococcus (χ^2= 43.27, p < 0.00) differed strongly by source.* ***Conclusion:*** *co-occurrence may reflect shared transmission routes, similar survival niches, or environmental/ecological overlap. This could facilitate the transfer of bacteria among humans, chickens, and the environment, some of which may be antimicrobial-resistant. There is, therefore, a need to promote best farm practices, including hand hygiene, to reduce bacterial transmission risks from animals to humans, and vice versain a shared environment.*

Introduction

The commensal bacteria *Escherichia* and *Enterococcus* are abundant in the gastrointestinal tract (GIT) of humans and animals, playing a significant role in maintaining normal mucosal immunity [1-3]. They are frequently isolated from human and animal faeces and commonly serve as bacteriological indicators of fecal contamination [4-6]. They may also be present outside their niche and get naturalized to an environment other than the GIT [7]. As commensals, *E. coli* and *Enterococcus* spp. act by inhibiting colonization of pathogens and enhancing nutrient supply to the body, hence promoting human and animal health; they rarely cause disease [6,8,9]. The environment is well endowed with the two commensal bacteria, as it serves as a reservoir for and of the dissemination of many bacteria [2]. The two bacteria are prime candidates for a One Health approach-based investigation of zoonotic bacterial infections, including antimicrobial resistance, due to their incidence, prevalence, and persistence throughout the interconnection spectrum [10,11]. However, although termed as commensal bacteria, *E. coli* and *Enterococcus* spp. could be opportunistic pathogens as well as carriers of resistance genes, playing a significant role in the development and spread of antimicrobial resistance (AMR) through different ecological avenues [12,13].

The gut microbiota of chickens is ubiquitous with the normal flora *E. coli* and *Enterococcus* species, which can evolve into pathogenic strains that are known to cause a variety of opportunistic infections and diseases [9]. Consequently, when shed from the chicken, the environment becomes a recipient and reservoir of bacteria (pathogens and potential pathogens for humans and animals), some of which are antimicrobial resistant [14,15]. Exchange of resistance genes may occur horizontally among bacteria (from commensals to pathogens), resulting in pathogens that are difficult to treat [6,7].

Direct human-livestock contact, ingestion of bacterial-contaminated food, and environmental dispersion are the three major routes of transmission of zoonotic infections [7,16,17]. Frequent human interaction with livestock at the farm level increases the chance of contracting

zoonotic bacterial infections [18]. Resistant enteric bacteria in humans, animals, and the environment have been shown to share some genotypic similarities [19] Multi drug resistant *E. coli* has been reported to be prevalent among poultry farm workers, chickens, and chicken environments [20]. Human hands are a major conduit for the spread of enteric pathogens, including *E. coli* and *Enterococcus* spp. from humans to the environment, and potentially act as a significant route of enteric pathogen exposure in humans. In low- and lower-middle-income countries, hand contamination has been shown to be equally common in rural and urban areas, underscoring the importance of hand hygiene in both contexts [21]. Contamination of human hands with *Enterococcus* spp. has been linked to the risk of respiratory infections in humans [6,22].

Anthropocentric practices such as excessive use of antimicrobials in poultry farming for treatment, prophylaxis, and as growth enhancers raise the risk of developing hotspots for the resurgence of zoonotic diseases as well as generating chains of antimicrobial resistance [23,24]. Misuse of antimicrobials may result in farmers being lax, exercising inadequate farm biosecurity and poor hygiene, thus impacting negatively on livestock disease prevention. This facilitates the transfer of bacteria among animals/chickens and humans, some of which may be zoonotic and antimicrobial-resistant. There is, therefore, a need to promote best farm practices, including hand hygiene, to reduce the spread of AMR from animals to humans and *vice versa.* through direct contact [18,20]. There are limited studies in sub-Saharan African countries on the impact of bacterial zoonoses at the animal-human-environment interface in poultry farming. Given the above data gaps, this study aimed to determine the prevalence of *E. coli* and *Enterococcus* spp. among chicken handlers, chickens, and chicken environs in Kiambu County, Kenya; ultimately aiming at promoting public health and raising awareness on bacterial transmission in poultry farming through the lens of a One Health approach.

Methods

Study design and site: a cross-sectional study was conducted between the months of June and September 2024, in three sub-counties, namely Kikuyu, Kabete, and Limuru of Kiambu County, Kenya. The study sites were chosen based on the area's high poultry populations and proximity to the isolation laboratory, the University of Nairobi (Figure 1). Both large- and small-scale poultry farms involving the three types of chicken (layers, broilers indigenous) were sampled. Large and small scales were defined as (501-5000 birds) and (50-500 birds), respectively [25].

Demographics of poultry farmers: a semi-structured questionnaire was employed to determine the age and gender of poultry farmers in Kiambu county, and the type of chicken they kept.

Study sample type and size: samples were taken from humans, animals, and the environment to reflect a One Health approach. They included; i) Human hand impressions on blood agar. ii) Chicken cloacal swabs. iii) Boot sock picks from the environment. Sample size (n) was determined following the formula by Thrusfield [26] based on an expected 50% prevalence (P), at a confidence level of 95% (Z), and a precision (d) of 10%.

$$n = \frac{Z^2 \, x \, P(1-P)}{d^2}$$

A total of one hundred and two (102) farms were sampled and yielded four hundred and eight (408). The category samples included One Health variables: handlers´ hands direct plating, chicken cloacal swabs, boot sock from interior floor, and exterior environments of the poultry house. The samples were processed for isolation of *E. coli* and *Enterococcus*, respectively, from each sample category.

Sampling procedure: research farms were chosen at random from the provided lists of farmers by the Kiambu Veterinary County Officer using a random number generator. Targeted farms included those that were willing to participate in the study and allow sample collection. The emphasis was on farmers engaging in large- or small-scale broilers, layers, and indigenous breeds of chicken. Samples were taken only from healthy chickens that were ready for market. Specifically, the layer breed chicken was considered after the production cycle (spent hens) because they are likely to gain entry into the food chain and, consequently, could potentially spread antimicrobial-resistant bacteria to humans through food.

Cloacal swabbing: to improve the accuracy and representativeness of the sample's bacterial recovery, for each farm, five chickens were sampled to create one pooled sample. Also, to ensure equal probability coverage and sample representation of the entire flock, on picking the five sample-chickens, the handler walked in a zigzag fashion inside the poultry house while maintaining the W pattern, and picked a healthy chicken at each intersection [27]. Thus, the five different cloacal swabs from five different healthy chickens, randomly picked per farm, were collected into Amies transport media to represent one sample for the farm; they were respectively labeled. The samples were then maintained in a cold chain and transported in a cooler box to the laboratory for further processing [28,29].

Hand plate sampling: hand plate sampling was done according to the methods by Singh S *et al.* and Espadale E *et al.* [30,31] with slight modifications. Blood agar, which is an enriched media that support the growth of fastidious and non-fastidious organisms, and also a differential medium, was used. After the chicken handlers had collected cloacal samples, they were requested to lightly press the surface of the already prepared blood agar plates with their dominant hand fingers and thumb to transfer any bacteria from the hand to the culture medium. The inoculated agar petri plates were inverted, safely secured in a petri-dish can holder aseptically, and transported to the microbiology laboratory for bacteria enumeration and isolation. The plates were incubated aerobically at 37°C for 24 hours (Annex 1). Bacterial colonies that grew on the plates were counted and recorded as colony-forming units. The count was recorded as >300 colony-forming units for plates with uncountable colonies.

Environmental sampling: boot socks were used for sampling of the interior poultry household and wider outdoor environment, and processed as described by Jones NR *et al*. Kintz E *et al*. [32,33]. Briefly, to sample the interior and exterior environments, the chicken handler wore boot socks (pre-dampened with 2ml of sterile saline) and separately walked randomly across the floor and the exterior surrounding of the poultry house. Firstly, a cover shoe was worn over the handler's shoe, followed by the sampling boot sock. The boot socks (two sets - exterior and interior) were then removed and placed separately in the pre-dampened plastic bag and labeled. The outer cover shoes were placed in biohazard bags and transported to the laboratory for decontamination and disposal. On the other hand, the sampling boot socks were separately placed in a sterile zip-lock bag and transported to the microbiology laboratory for further processing under a cold chain.

Sample handling and transportation: all samples were clearly labeled and transported with their corresponding details to the microbiology laboratory, Faculty of Veterinary Medicine, at the University of Nairobi, for further processing. To maintain the integrity of the samples, the swabs and boot socks were transported under a cold chain to the laboratory for bacterial enumeration and isolation within the same day, minimizing all possible delays [28,29]. Arrangements such as minimal batch sampling and strategic region-by-region sampling were made to ensure that the samples arrived in the laboratory not more than four hours after collection. The inoculated hand plates were clearly labeled, and put in petri-dish

holder canisters before the onset of room incubation while on transit to the laboratory for further incubation at 37°C within the same day.

Preliminary processing of the environmental boot socks for microbial isolation: on arrival at the laboratory, 100ml of single-strength buffered peptone water (BPW) was added to the bag containing the pair of boot socks, followed by hand palpating (massaging for one minute) [32,33]. Exactly, 10ml of the BPW supernatant was transferred into a sterile universal tube and incubated overnight at 37°C, for further isolation of *E. coli* and *Enterococcus*.

Preliminary processing of the cloacal swabs for microbial isolation: the five swabs collected per farm were pooled into 10 ml of BPW to constitute one homogenate sample. This was then further incubated overnight at 37°C, for further isolation of *E. coli* and *Enterococcus*.

Preliminary handling of the inoculated hand plates for microbial isolation: total bacterial counting was done and recorded on all the blood agar plates previously incubated at 37°C. This was followed by harvesting all the bacterial growth using a sterile wire loop into 10 ml of BPW to constitute one homogenate sample per farm. The samples were then processed for further isolation and identification of *E. coli* and *Enterococcus*.

Microbial isolation and characterization of *E. coli* and *Enterococcus*: according to accepted laboratory protocol [34], all bacterial culture, isolation, and phenotypic characterization (Annex 1) were carried out at the University of Nairobi's microbiology laboratory, Department of Veterinary Pathology, Microbiology, and Parasitology, Faculty of Veterinary Medicine.

Isolation and characterization of *E. coli*: for isolation of *E. coli*, a loopful of inoculum from the overnight BPW culture from each sample category was aseptically streaked onto MacConkey agar plates and incubated aerobically at 37°C for 24 hours. The primary mixed culture was sub-cultured on a clean MacConkey plate to achieve distinct pure colonies. Gram stain was performed on a single distinctive lactose fermenter colony, followed by biochemical testing of Indole, Methyl red, Voges Proskauer, and Citrate (IMVC), Triple Sugar Iron (TSI), and urease. A selective medium, Eosin Methylene Blue (EMB), was used to confirm the identity of *E. coli* as described by [34].

Isolation and characterization of *Enterococcus*: to optimize the isolation of *Enterococcus* spp. precisely 1 ml of overnight BPW culture broth from each sample type was inoculated into 10 ml of *Enterococcus* pre-enrichment medium, Azide Dextrose Broth, and incubated aerobically at 37°C for 24 hours. This was followed by streaking a loopful of the pre-enriched inoculum into the *Enterococcus* selective medium Slanetz & Bartley and incubation for 24 hours aerobically at 37°C. Colonial morphology was observed, and distinct maroon colonies were Gram-stained and sub-subcultured onto nutrient agar. Biochemical tests, including catalase and bile aesculin, were performed on pure culture colonies.

Statistical analysis: all the data was cleaned, validated, and imported into Microsoft Excel, where descriptive and inferential statistical analysis were performed using R version 4.2.2. The prevalence of two bacteria was summarized as counts and percentages for each source. Association and differences in prevalence across sources were assessed using the Chi-square test of independence, with Fisher´s exact test applied when expected cell counts were <5. Strength of association was quantified using the phi coefficient (ϕ). Logistic regression models were fitted to evaluate the association between source and the odds of detecting the two bacteria and their co-occurrence. Chicken was set as the reference category, and odds ratios (ORs), 95% confidence intervals (CIs), standard errors, and p-values were reported. Model fit was assessed using the pseudo-R^2statistic. All tests were two-tailed. A p-value <0.05 was considered statistically significant. Heatmaps were utilized to illustrate

the distribution of *E. coli* and *Enterococcus* spp. across these variables, the prevalence data were organized into a matrix format. Bubble plots were also constructed to depict the relationship between bacterial presence, with bubble sizes proportional to prevalence counts. In the spatial analysis section, a combination of geospatial and cartographic techniques was used to visualize the study area within Kiambu County, Kenya. This included detailed mapping of sub-county boundaries and specific sampling locations. Visualizations were created using the *ggplot2, sf, geodata, and ggspatial* R packages [35]

Results

Bacterial colony counts among chicken handlers: heavy and confluent bacterial growth was present on all the inoculated human-hand-inoculated blood agar plates. The colonies could not be counted among all the one hundred and two (102) chicken handlers sampled and were recorded as being more than 300 colonies [36]. Furthermore, this study established involvement in poultry keeping with respect to gender and age. The handlers sampled were aged between 18 and greater than 75 years; there was variation in involvement with respect to gender and age. The majority were female, 73/102 (71.6%), compared to males 29/102 (28.4%). The largest age group was 45-54 years (29.1%), followed by 35-44 years (24.3%) and 65-74 years (15.5%). Smaller proportions were observed among participants aged 18-24 years (3.9%), 25-34 years (7.8%), and >75 years (1.9%). An interesting trend was observed where poultry farmers between the age brackets of 65 to 74 were only females 16/102 (15.5%). In a separate instance, 7/102 (6.9%) comprising young males aged between 25 and 34, were engaged in poultry production, compared to 1/102 (0.98%) females of the same age bracket (Table 1, Figure 2).

Prevalence of *E. coli* across chicken handlers, chickens, and chicken environments: a high prevalence of *E. coli* was observed, with 99% (101/102) in chicken samples and the surrounding external environment, and 100% (102/102) on the poultry house floor (internal environment). Additionally, a prevalence of 10.8% (11/102) was identified on the hands of chicken handlers (Table 2, Figure 3). Chi-square test revealed the prevalence of *E. coli* (χ^2= 331.22, p < 0.001) differed strongly by source.

Prevalence of *Enterococcus* spp. across chicken handlers, chicken, and chicken environments: the prevalence of both *Enterococcus* spp. in chicken samples and the surrounding external poultry environment was 98.0% (100/102). Within the internal poultry house environment, *Enterococcus* spp. was found at 99.0% (101/102) and 81.4% (83/102) on the hands of chicken handlers (Table 2, Figure 4). Chi-square test revealed the prevalence of *Enterococcus* (χ^2= 43.27, p < 0.00) differed strongly by source.

Prevalence of *E. coli* and *Enterococcus* spp. across chicken handlers, chicken, and chicken environments: *E. coli* and *Enterococcus* were highly prevalent in chicken and environmental samples, with greater than 95% positivity, while only 9.8% of human pathogens were positive for both pathogens (Table 2, Figure 5). Logistic regression confirmed that humans were significantly less likely to carry *E. coli* on their hands (OR ≈ 0.004, 95% CI: 0.001-0.012, p <0.001) and *Enterococcus* (OR ≈ 0.087, 95% CI: 0.014-0.313, p = 0.001) compared to chicken (Table 2). Similarly, humans had lower odds of co-occurrence of both pathogens (OR ≈ 0.004, 95% CI: 0.001-0.013, p <0.001). In contrast, environmental samples (outside and inside) were not significantly different from chicken and had wider CI due to almost all being positive (Table 2).

Further co-occurrence analysis revealed a positive association between *E. coli* and *Enterococcus*. Among all samples, 76 of 95 (80%) were negative for *E. coli* but still positive for *Enterococcus*. It was also noted that 309/313 (98.7%) of *E. coli* positive samples were also *Enterococcus*-positive (Table 3). Chi-square test confirmed this association (χ^2=

44.57, p < 0.0001), and phi coefficient showed a moderate positive association (ϕ = 0.34). *Enterococcus*-positive samples had 19.3 times higher odds of *E. coli* detection compared to *Enterococcus*-negative samples (95% CI: 6.4-58.4, p <0.0001). The reverse model produced the same odds ratio, indicating that *E. coli* positive samples were also 19.3 times more likely to harbor *Enterococcus* (95% CI: 6.4-58.4, p <0.0001) (Table 3).

Discussion

The current study investigated the prevalence of *Enterococcus* spp. and *E. coli* across key One Health factors in poultry environments. Across all three variables investigated, the overall sample-level prevalence of the commensal bacteria *E. coli* and *Enterococcus* was high (above 80%), with a notable and significant difference in the prevalence of E. coli on chicken handlers' hands, at 10.8%. As mentioned earlier in the introduction, the commensal bacteria, *E. coli* and *Enterococcus* are abundant in the gastrointestinal tract, with their high prevalence of isolation in this study.

Though crucial for preserving healthy mucosal immunity and being excellent sources of probiotics, they could be potential pathogens and carriers of antimicrobial resistance genes, significantly contributing to the establishment and dissemination of antibiotic resistance via many environmental pathways [12,13]. Of particular concern is the consumption of large quantities of *Enterococcus* organisms in food, especially as probiotics, due to their high effective gene transfer ability and as potential donors of resistance and virulence genes to other pathogenic and non-pathogenic bacteria in the gastrointestinal tract [37]. As reported by [38], antimicrobial-resistant microorganisms are prevalent in Kenyan environments and animals, and present a risk of transmission to humans. *Enterococcus* has been associated with nosocomial infection and hospital outbreaks propelled by its innate ability to tolerate environmental stresses, including antimicrobials [39-41]. Research has also reported possible direct transmission of *Enterococcus*organisms through hands and indirectly through environmental surfaces [41]. This implies that hand hygiene and environmental safety are fundamental in the control and prevention of enterococcal infections.

Blood agar plates inoculated by the chicken handlers´ hands were marked with too many colonies and an apparent confluent growth, hence regarded as being >300 CFU, signifying heavy bacterial contamination on the participant´s hands. The range of countable colonies on a plate, as commonly accepted, is 250 to 300 CFU [36]. The method assumes that each colony arises from one bacterial cell and is counted to give a count in colony-forming units (CFU). Isolation of *Enterococcus* and *E. coli* in the inoculated plates suggests possible contamination of hands with the two enteric bacteria, among other bacteria. It also suggests the possible transfer of bacteria from chicken to humans within poultry environments. This collaborates with a previous study showing that, globally, hands are commonly contaminated with enteric pathogens and faecal bacteria [21].

Interestingly, findings from this study demonstrate that in contrast to the relatively low frequency of *E. coli* (10.8%), the prevalence of *Enterococcus* isolated from the hands of chicken handlers was considerably high (81.4%). A possible explanation for this might be the adaptability and versatility of *Enterococcus* to a wide range of environments, including the ability to survive outside their niche [42]. Another important finding is that the heavy hand contamination was across both genders and age groups - youth to elderly, showing that their farm practices were similar. This observation underscores the importance of hand hygiene, with the need to target all genders and age groups.

Enterococcus and *E. coli* were also prevalent in the cloaca of the chicken, which was consistent with the findings of Gupta CL *et al.* [43] and Ribeiro J *et al.* [9]. The most striking observation was the high

prevalence (99%) of the two commensal bacteria in chicken; they have the potential of harboring antimicrobial resistance genes that could be transferred to humans either through direct contact or indirectly through the environment, when shed from the chicken [9,43]. The high bacterial prevalence reported from chicken cloaca in this investigation is in tandem with a report of 100% isolation of *E. coli* from the cloaca of healthy broiler chickens in Bangladesh [44].

This study showed widespread contamination with *Enterococcus* and *E. coli* in both the interior floor and exterior surroundings of the poultry house, implying that cross-contamination may occur in and out of chicken environs, exposing humans and animals to bacteria from the environment. *Enterococcus* has been used as an excellent indicator of environmental contamination and a measure of hygiene in food production [37,41]. This implies that *Enterococcus* is crucial in reporting levels of contamination in the environmental and food hygiene. Mostly, in animal production, the antimicrobial resistance described in commensal bacteria *Enterococcus* and *E. coli* is due to anthropocentric influences such as antimicrobial use in raising food animals, signifying that the two bacteria have a greater risk to development of developing antimicrobial resistance [45].

The present study raises the possibility that bacterial transfer may occur between humans, animals, and the environment, supporting the findings of Zaheer R *et al.* and Collignon PJ *et al.* [7,12], where *Enterococcus* species isolated showed clear correlations throughout the One Health spectrum. Studies have shown that fecal content from healthy chickens may harbor virulent and pathogenic strains of *E. coli* that can infect humans through direct contact or indirectly through consumption of chicken products [46]. Further on, these results corroborate the ideas of Mencía-Ares O *et al.* [45], suggesting that commensal *Escherichia coli* and *Enterococcus* organisms may be suitable on-farm bio-indicators for assessing elements that lead to the development of antimicrobial resistance across the human-animal-environment interface. In agreement with the findings of Hedman HD *et al.* [24], chicken and chicken environs increase the chances of antimicrobial resistance and recurrence of zoonotic diseases during interaction with humans.

In accordance with the present results, previous studies have demonstrated that infection prevention and control (IPC), such as hand hygiene, farm biosafety-biosecurity, are crucial strategies in the fight against zoonotic including bacterial-related infections [47,48]. A One Health strategy requires examining the interconnectedness between humans-animals, and the environment [10,12,45]. The findings of this study broadly support the work of other studies [10,45,49] in reporting the usefulness of commensal *Enterococcus* and *E. coli* in monitoring public health, environmental safety, and antimicrobial resistance surveillance. This is due to their high prevalence in humans, animals, and the environment, including their ease of laboratory isolation with minimal cost [13,40].

Conclusion

This study set out to establish the prevalence of *E. coli* and *Enterococcus* in chicken, chicken handlers´ hands, and the chicken environments. The results indicate a high prevalence of the two bacteria across the three variables investigated, some of which may be antimicrobial-resistant. Co-occurrence may reflect shared transmission routes, similar survival niches, or environmental/ecological overlap. There is, therefore, a need to promote best farm practices, including hand hygiene, to reduce the transmission risk of bacteria from animals to humans and *vice versa*. However, further research is needed to establish the virulence of *Enterococcus*, antimicrobial susceptibility patterns, and genetic relatedness of the *Enterococcus* and

E. coli isolated from humans, animals, and the environment.

What is known about this topic

- *Antimicrobial resistance is a rising global threat to human and animal health;*
- *Commensal bacteria Enterococcus spp. and E. coli are potential opportunistic pathogens and reservoirs of antimicrobial resistance genes;*
- *Antimicrobial use in both human and animal health is a major driver of AMR.*

What this study adds

- *Co-detection of enteric bacteria, particularly Enterococcus spp. and E. coli on human hands;*
- *Transmission risk: Direct human-chicken contact during handling facilitates bacterial transmission;*
- *Interventions are needed to inform directions in hand hygiene and public health promotion among poultry farmers.*

Competing interests

The authors declare no competing interests.

Authors' contributions

Ann Munene: conceptualization, methodology, validation, investigation, data collection, investigation, project administration, and writing - original draft. Lilly Bebora: conceptualization, methodology, validation, supervision, writing - review and editing. Peter Mwangi: data analysis, writing, review, and editing. Christine Mbindyo: conceptualization, methodology, validation, supervision, writing - review and editing. Kelvin Ndeto: data collection and investigation. Charity Gathenya: investigation. George Ndimbu: investigation. John Maingi: conceptualization, methodology, validation, supervision, writing, review, and editing. All the authors have read and agreed to the final version of this manuscript.

Acknowledgments

The authors acknowledge all the poultry farmers who participated in the study. A special thanks to the sub-county veterinary officers and the livestock production officers of Kiambu County, who granted access to the sub-counties and made it possible for the study to be conducted. We express gratitude to the Microbiology team at the Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi. Special acknowledgement to Lydia Maina, Risper Njoki, and Evelyne Moraa for their support in the laboratory work.

Tables and figures

Table 1: age and gender of poultry farmers

Table 2: prevalence of *E. coli* and *Enterococcus*, their co-occurrence, and logistic regression by sample source

Table 3: association between *E. coli* and *Enterococcus*

Figure 1: a Kenyan map of study sites: Kamuguga, Kerwa, Kirimiti, Muguga, Ngecha, Sigona, and Uthiru wards in Kiambu County

Figure 2: distribution of the chicken handlers sampled, with respect to age and gender

Figure 3: prevalence of *E. coli* across chicken, chicken handlers' hands, and chicken environments

Figure 4: prevalence of *Enterococcus* across chicken, chicken handlers, and chicken environments

Figure 5: comparison of prevalences of *E. coli* and *Enterococcus* isolates across chicken handlers, chicken, chicken environts; Ec absent- *E. coli* absent, Ec present- *E. coli* present, Ent absent-*Enterococcus* absent, Ent present-*Enterococcus* present

Annex

Annex 1: flow chart showing processing and isolation of *E. coli* and *Enterococcus* spp. (PDF 214 KB)

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Table 1: age and gender of poultry farmers

Age	**Female, n (%)**	**Male, n (%)**	**Total, n (%)**
18-24	2 (2.7)	2 (6.9)	4 (3.9)
25-34	1 (1.4)	7 (24.1)	8 (7.8)
35-44	17 (23.3)	8 (27.6)	25 (24.3)
45-54	23 (31.5)	7 (24.1)	30 (29.1)
55-64	13 (17.8)	4 (13.8)	17 (16.5)
65-74	16 (21.9)	0 (0.0)	16 (15.5)
>75	1 (1.4)	1 (3.4)	2 (1.9)
Total	**73 (100)**	**29 (100)**	**102 (100)**

Table 2: prevalence of *E. coli* and *Enterococcus*, their co-occurrence, and logistic regression by sample source							
Prevalence and co-occurrence							
Source	**N**	***E. coli* (+)**	***E. coli* (%)**	***Enterococcus* (+)**	**Enterococcus (%)**	**Both (+)**	**Both (%)**
Chicken	102	99	97.1%	100	98.0%	98	96.1%
EnvIn	102	102	100%	101	99.0%	101	99.0%
EnvOut	102	100	98.0%	101	99.0%	100	98.0%
Human	102	11	10.8%	83	81.4%	10	9.8%
Logistic regression (chicken as reference)							
Outcome	**Comparison (vs Chicken)**	**OR**	**95% CI**	**Std error**	**p-value**		
E. coli	EnvIn	~NA*	Unstable est.	-	0.992		
	EnvOut	3.06	0.39-62.4	1.16	0.336		
	Human	0.004	0.001-0.012	0.667	<0.001		
Enterococcus	EnvIn	2.02	0.19-43.9	1.23	0.569		
	EnvOut	2.02	0.19-43.9	1.23	0.569		
	Human	0.087	0.014-0.313	0.758	0.001		
Co-occurence	EnvIn	4.12	0.60-81.4	1.13	0.209		
	EnvOut	2.04	0.39-15.0	0.878	0.416		
	Human	0.004	0.001-0.013	0.609	<0.001		
E. coli- Escherichia coli; EnvIn- interior environment of poultry houses; EnvOut- surrounding environment of poultry houses; NA-not applicable; unstable est.- unstable estimate							

Table 3: association between *E. coli* and *Enterococcus*			
2×2 contigency table			
	***Enterococcus* (-)**	**Enterococcus (+)**	**Total**
E. coli (-)	19	76	95
E. coli (+)	4	309	313
Total	23	385	408
Measure of association			
Statistic	**Value**	**95% CI**	**p-value**
Chi-square (1 df)	44.6	–	<0.0001
Odds ratio (*E. coli* ↔, *Enterococcus*)	19.3	6.4-58.4	<0.0001
Phi coefficient (ϕ)	0.34 (moderate positive)	–	–
E. coli- Escherichia coli; CI- confidence intervals			

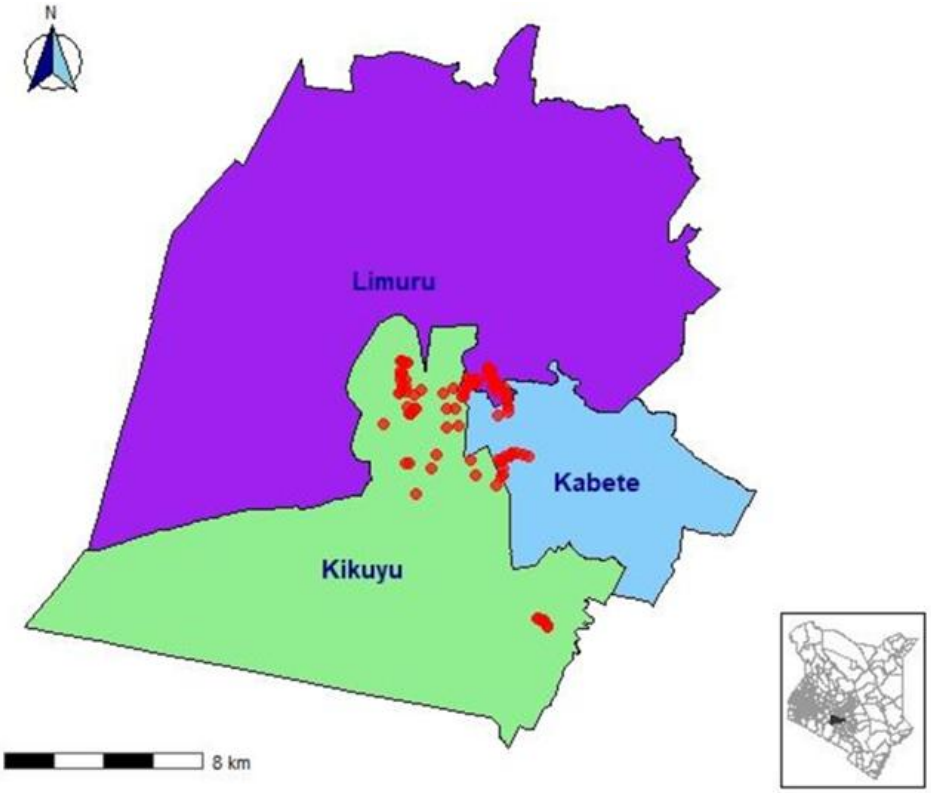

Figure 1: a Kenyan map of study sites: Kamuguga, Kerwa, Kirimiti, Muguga, Ngecha, Sigona, and Uthiru wards in Kiambu County

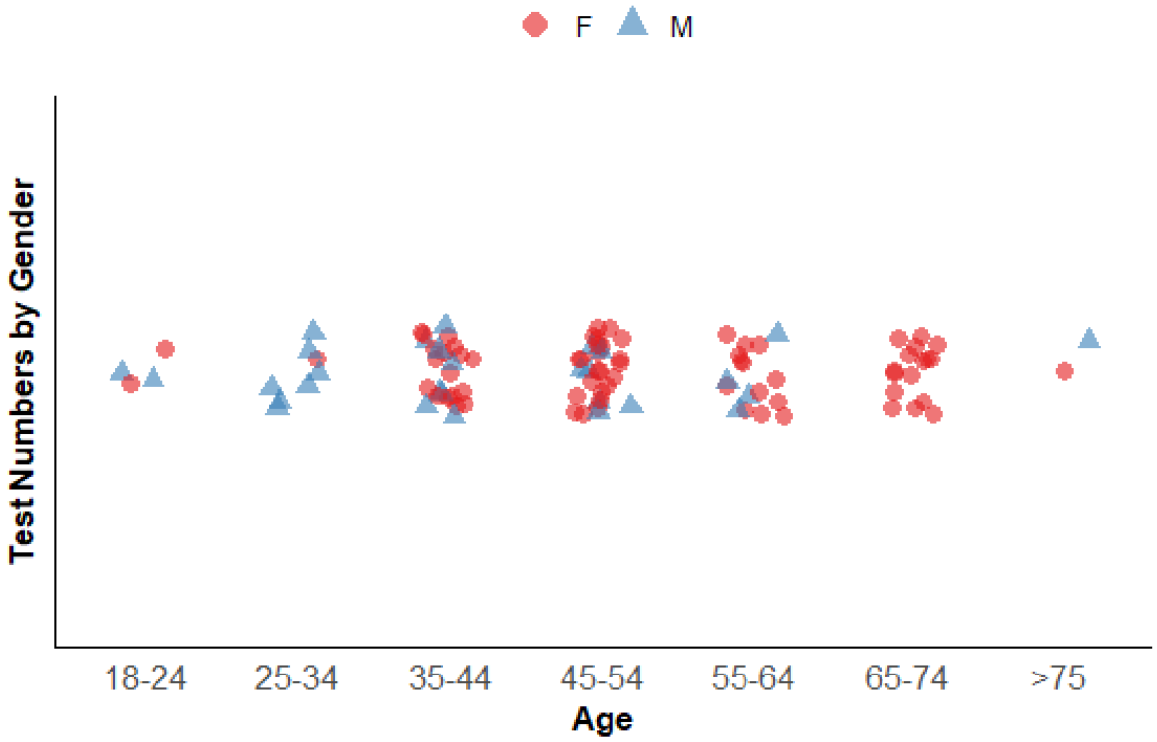

Figure 2: distribution of the chicken handlers sampled, with respect to age and gender

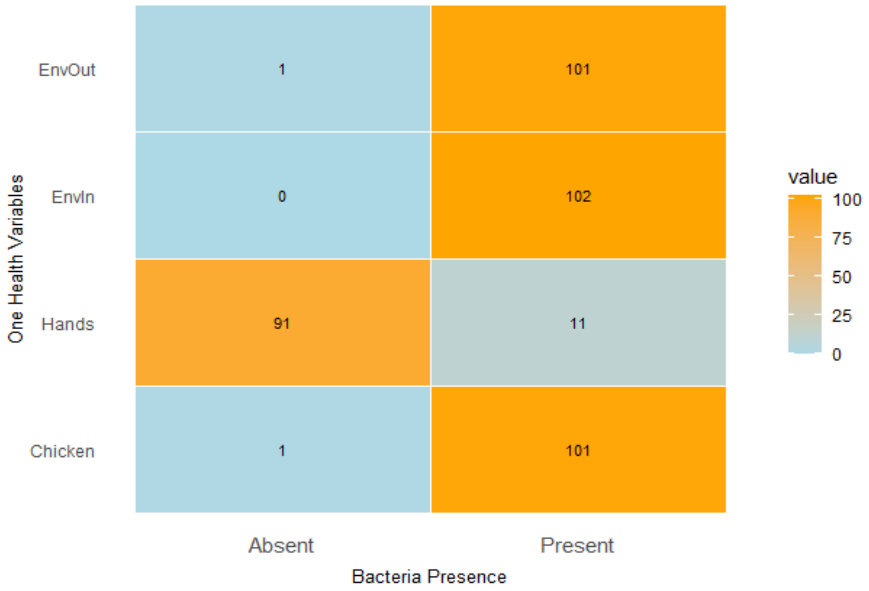

Figure 3: prevalence of *E. coli* across chicken, chicken handlers' hands, and chicken environments

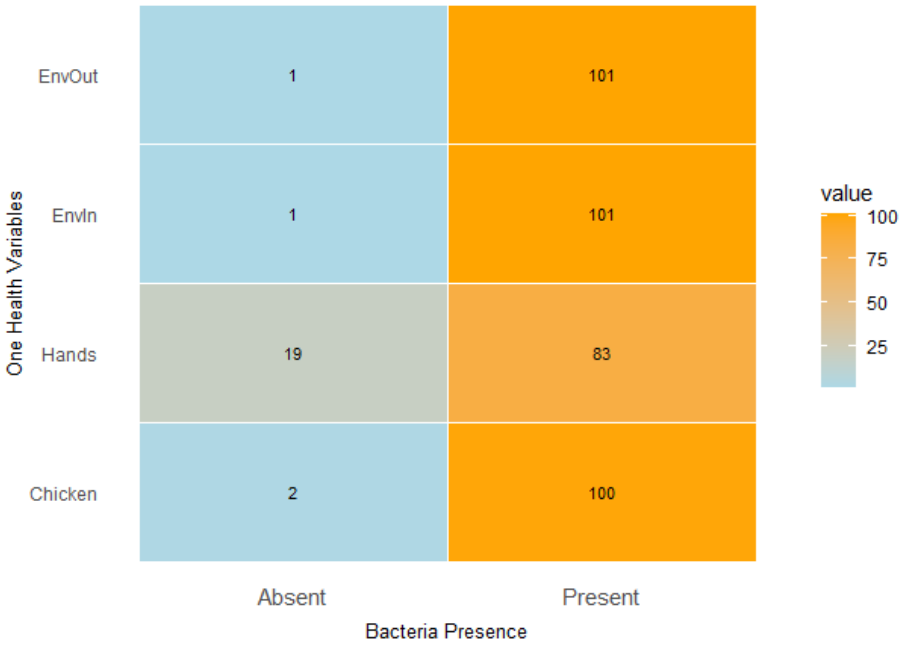

Figure 4: prevalence of *Enterococcus* across chicken, chicken handlers, and chicken environments

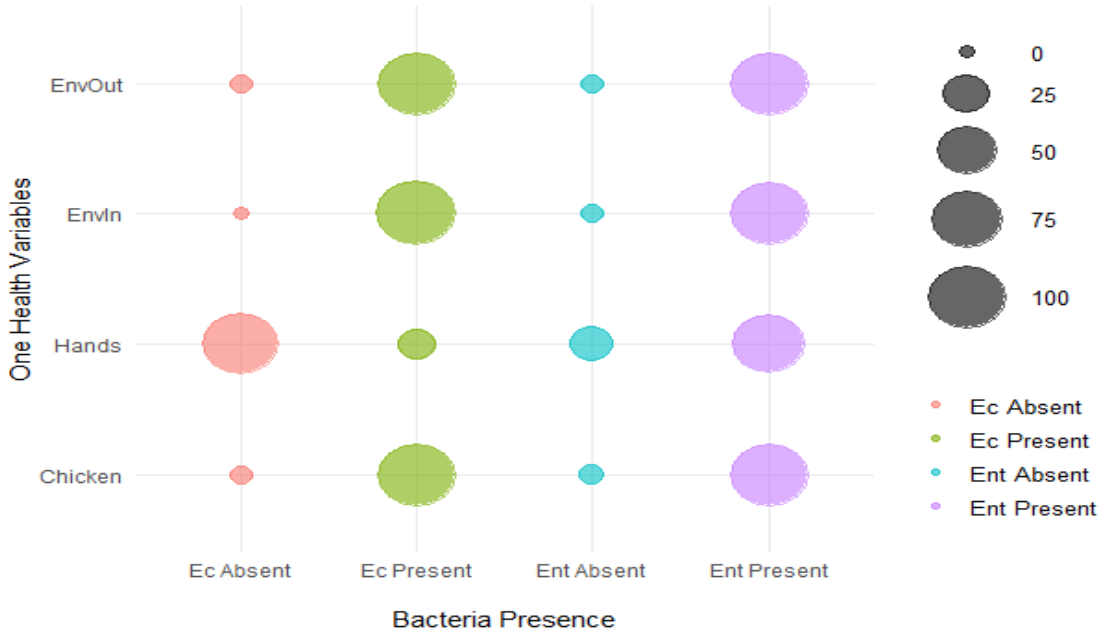

Figure 5: comparison of prevalences of *E. coli* and *Enterococcus* isolates across chicken handlers, chicken, chicken environts; Ec absent- *E. coli* absent, Ec present- *E. coli* present, Ent absent-*Enterococcus* absent, Ent present-*Enterococcus* present