ANTIBIOTICS SUSCEPTIBILITY PROFILE OF *ESCHERICHIA COLI* ISOLATED FROM POULTRY ENVIRONMENT

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ABSTRACT

The rise in the prevalence of antibiotic-resistant pathogenic bacterial species poses an escalating challenge globally, particularly in developing countries. The extensive utilization of antimicrobials in poultry farming raises apprehensions about the emergence of multidrug-resistant microbial species that could be transmitted to humans. The present study assesses the antimicrobial susceptibility profile of *Escherichia coli* isolated from various poultry environments, including fecal, soil, feed, and water samples, in the Akoko areas of Ondo State. Fifty-three strains of *Escherichia coli* were isolated from the samples obtained from the poultry environments based on their cultural characteristics on Eosin-methylene Blue agar, Gram staining reaction, morphological and biochemical attributes. The susceptibility of isolates to specific antibiotics was examined utilizing the adapted Kirby-Bauer disc diffusion technique in accordance with CLSI recommendations. The majority of the isolates exhibited resistance to tetracycline which is a commonly used antibiotic in the region. However, gentamicin, ceftriaxone, ciprofloxacin, amikacin and chloramphenicol demonstrated effectiveness against a significant proportion of *E. coli* isolates. Findings from this investigation indicate that environments within poultry facilities may function as possible sources for antibiotic-resistant *E. coli*, posing a significant public health concern. Hence, it is crucial to focus on the production of safe poultry and high-quality poultry products, as well as regulate the use of antibiotics in poultry farming.

Keywords: Antibiotic resistance, *Escherichia coli*, Poultry environment, Poultry products, Antimicrobials

Introduction

The development of antibiotic resistance presents a critical challenge, not only in terms of healthcare but also economically (Banin *et al*., 2017, Ahmad & Khan, 2019). As resistant bacteria proliferate, there's a growing fear that treating infections will become more challenging and costly (Sommer *et al*., 2017). This threat emphasizes the urgent need for sustainable strategies to address antibiotic resistance and the development of alternative approaches to combat bacterial infections. Surveillance is vital to the management of antibiotic resistance (Tacconelli *et al*., 2018). Access to current and local data on resistance patterns is invaluable in assessing the effectiveness and appropriateness of antibiotics (WHO, 2018). This information helps healthcare providers make informed decisions regarding antibiotic prescriptions, ensuring that the chosen antibiotics are still effective against prevalent pathogens in their specific regions. Surveillance data also aids in the formulation of antibiotic policies and

guidelines, guiding efforts to combat resistance effectively while optimizing antibiotic use (Yasmin *et al*., 2022).

Food animals and the places where they're raised harbour resistant bacteria as well as resistance genes. These can transfer to humans either through direct contact between animals and humans, indirectly through the food production chain (Adelowo *et al*., 2014, Bennani *et al*., 2020), or through the spreading of animal waste on land (Khan *et al*., 2020). Antimicrobial agents are frequently utilized in Nigeria's livestock farming, with common practice involving their addition to both water and feed sources (Jibril *et al*., 2021). Such a practice can confer a selective advantage, resulting in a rise of the prevalence of antibiotic-resistant bacteria either from the animals, their excretions, or the adjacent environment (Adelowo *et al*., 2014).

Escherichia coli (E. coli) is typically part of the natural microflora in warm-blooded animals, including poultry (Sarba *et al*., 2019). However, in individuals with reduced immunity, or when gastrointestinal defenses are compromised, even what's typically seen as a non-pathogenic strain of *E. coli* can lead to infections in humans, poultry, and other animals. Globally, there's a prevalence of multidrug-resistant strains of *E. coli* in human and in animal isolates (Moffo *et al*., 2021). Nonpathogenic *E. coli* with multiple drug resistance found in the intestine is likely a substantial reservoir of resistance genes (Ravi *et al*., 2014). Moreover, there are documentations suggesting drug-resistant *E. coli* originating from animals can establish colonisation in the human intestine (Hassan *et al*., 2014). This can lead to illness, fatalities, and increased healthcare expenses. Hence, this study aims to isolate *Escherichia coli* from various sources in the poultry environment in Akoko, Southwest Nigeria and assess their susceptibility to selected antibiotics.

Experimental

Sample collection: Samples were collected from selected poultry farms in Akoko Area of Ondo State Nigeria. Four different samples; feed, water, fecal, and soil samples were collected using sterile spatula. The spatula used was sterilised with 70% ethanol and used to pick samples in selected poultry into sterile McCartney bottles. The samples were transferred to the laboratory right after being collected which was within 3hrs.

Isolation of Escherichia coli: Isolation and identification of bacteria isolate were carried out in the laboratory using standard procedures for aerobic bacteria. For the detection of *Escherichia coli*, 1 g of the fecal, feed, soil or 1 mL of water samples collected from poultry environment were measured into sterile bottles containing 9 mL of sterile distilled water aseptically. The suspension underwent thorough vortexing before transferring 1.0 mL into 9.0 mL of sterile Tryptic Soy Broth (TSB) (Oxoid Ltd, Basingstoke, Hampshire, England) for the purpose of enrichment. The inoculated TSB was incubated for 24 hours at 37^{o}C. After 24 hours of incubation, the broths were vortexed vigorously before 0.1 mL from each sample was spread on the surface of already prepared Eosin methylene blue agar (EMB) (Oxoid Ltd, Wade Road, Basingstoke, Hampshire, RG24 8PW, United Kingdom) in triplicates. Inoculated plates were incubated for 24 hours at 37^{o}C to allow the growth of the bacteria. Colonies showing the cultural morphologies of *E. coli* i.e., 'metallic green sheen' on EMB agar were sub-cultured on fresh media to obtain pure culture of the colonies of interest. Colonies of Isolates were

further sub-cultured on Sorbitol MacConkey agar (Oxoid, England) to distinguish *E. coli* strain not capable of fermenting sorbitol which is characteristic of *E. coli* 0157.H7. The plates were then examined for growth, morphological and biochemical characteristics of selected bacteria colonies were also done to further confirm the identity of the isolates.

Antibiotics Susceptibility Test of Isolate: The sensitivity of bacteria isolates to selected antibiotics was tested using the Kirby-Bauer diffusion method. Commercially available pre-impregnated antibiotics disc containing Ceftazidime (30µg), Gentamicin (10µg), Meropenem (10µg), Ceftriaxone (30µg), Tetracycline (30µg), Cefotaxime (30µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Amikacin (30µg), Cefuroxime (30µg) were used for the test.

Fresh overnight growth of the bacteria isolates were prepared in LB broth (Oxoid, UK). Cells were recovered from the overnight culture by centrifugation at X8,000g for 5 minutes. Recovered cells were resuspended in PBS and washed by centrifugation. The washed cells were resuspended in PBS and the cells were adjusted to a final concentration of 10^8 CFU/mL which is equivalent to 0.5 McFarland standard. The prepared inoculum was swabbed on the surface of the already prepared Mueller Hinton agar (Oxoid, UK). The seeded plates were allowed to dry for 15 minutes before the antibiotics disk were aseptically placed on the agar. These were incubated at 37°C for 24 hours after which they were examined for zone of inhibition. The diameter of the clear zones of inhibition around each disk were measured with a ruler. Depending on the diameter of the zone of inhibition, the isolates were either categorised as susceptible, intermediate or resistant based on CLSI (2019) standard.

Results

This study evaluated the prevalence and antibiotic susceptibility profile of *Escherichia coli* in poultry environment in Akungba Akoko. *E. coli* isolates were identified based on their cultural characteristics on Eosine Methylene blue. In addition to the morphological characteristics, the Gram stain reaction, cellular morphology and selected biochemical characteristics of the isolates were also evaluated. A total of 53 were identified as *E. coli*. Figure 1 illustrates the percentage of samples that tested positive for *E. coli*. Fecal samples exhibited the highest frequency of positive results for *E. coli*, followed by soil samples and water samples, whereas feed samples showed the lowest incidence of positive results for *E. coli*. The antibiotic susceptibility pattern of the fifty-three isolates is shown in Figure 2. Gentamicin and Ceftriaxone were active against most of the isolates while tetracycline showed activity against the least number of isolates. Some of the isolates showed resistance to multiple antibiotics.

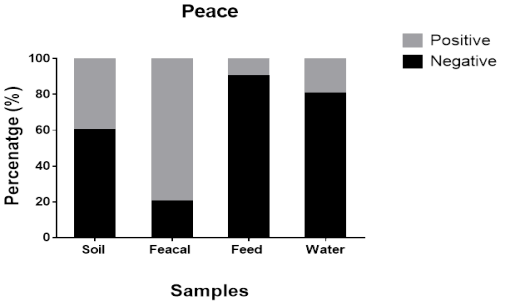

Fig. 1: Percentage of samples positive with *Escherichia coli*

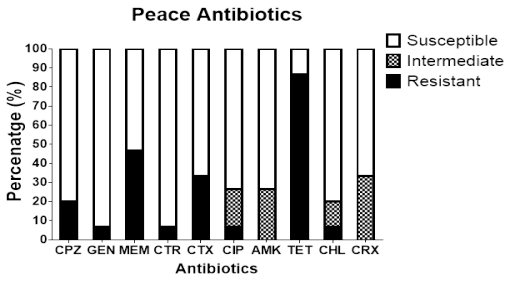

Fig. 2: Percentage of isolates showing resistance, intermediate resistance and susceptible to selected antibiotics (Ceftazidime (CPZ), Gentamicin (GEN), Meropenem (MEM), Ceftriaxone (CTR), Cefotaxime (CTX), Ciprofloxacin (CIP), Amikacin (AMK), Tetracycline (TET), Chloramphenicol (CHL), Cefuroxime (CRX)

Discussion

Antibiotic resistance has become a significant threat to public health in the 21st century, as indicated by global surveillance data (Puvača & de Llanos Frutos, 2021). The unrestricted use of antibiotics is leading to extensive antimicrobial resistance, a matter that has garnered significant national and international concern. It is therefore imperative to maintain continuous monitoring of antibiotic-resistant bacteria to effectively combat the global dissemination of such strains. Animal production facilities such as poultries are known hotspots for the emergence of antibiotic resistance due to indiscriminate use of antibiotics more so in developing countries where regulations against such practice are either weak or not in existence. In this study, the antibiotic susceptibility pattern of *Escherichia coli* isolated from various samples collected from poultry environments was determined.

Certain isolates obtained from various samples were identified as *E. coli* through assessment of their cultural, cellular morphology, and biochemical characteristics. Pathogenic strains of *Escherichia coli* are common human pathogens that can be acquired from animal sources (Bélanger *et al*., 2011, Ferens & Hovde, 2011). Poultry products have been variously implicated as sources of human pathogenic *E. coli* strains (Mora *et al*., 2013, Stromberg *et al*., 2017). The fecal samples were most positive for *E. coli* compared to other samples collected. A similarly elevated prevalence of *E. coli* in fecal samples, in comparison to other sample types, has also been reported (Panchal *et al*., 2020, Mondal *et al*., 2023). Fecal samples are ideal for the evaluation of enteric pathogens like *E. coli*. Fecal material contaminated with *E. coli* can potentially lead to the spread of *E. coli* to the poultry environments which can lead to wider environmental contamination and thus creating a human exposure risk. Likewise, this can lead to the contamination of poultry products such as eggs which can also result in human infection.

Other samples collected from the poultries studied likewise showed *E. coli* contamination although with a lower incidence rate compared to the fecal samples. This may be caused by contamination with fecal materials through unhygienic practices in the poultry facilities studied. *E. coli* contamination of the feeds samples may also result from the contamination of the materials used in their formulations. Locally available cheap protein sources such as locally processed fish wastes are commonly used in local formulations of chicken feeds. This has been documented as a significant cause of bacterial contamination in poultry feeds (Okoli *et al*., 2006, Hoque *et al*., 2019).

E. coli strains can employ diverse mechanisms to resist antibiotic thus making *E. coli* infections harder to treat. The *E. coli* isolated in this study showed variable sensitivity to the different antibiotics tested. The highest

resistance was found against tetracycline, followed by meropenem while ceftazidime, gentamicin and cefotaxime were active against most of the *Escherichia coli* isolates. Previous studies have shown widespread resistance to tetracycline in *E. coli* isolated from different sources in Nigeria (Adelowo *et al*., 2014, Adenipekun *et al*., 2015). The widespread resistance to tetracycline in *E. coli* isolates in Nigeria may be because of the widespread abuse of the antibiotics. Tetracycline is one of the most misused antibiotics in Nigeria (Olatoye & Basiru, 2013, Jamiu *et al*., 2016, Alhaji & Isola, 2018). Bissong *et al*. (2023) have also documented extensive resistance to Meropenem in *E. coli* isolates.

Most of the *Escherichia coli* isolates in this study exhibited multiple resistance to more than 2 antibiotics. This observation is similar to the report of multiple drug-resistant strains of *E. coli* isolated from poultry sources in other parts of the world (Noppon *et al*., 2018, Rahman *et al*., 2020). Such high incidence of drug resistance isolates may be attributed to indiscriminate use of antibiotics in poultry farming gents, which may ultimately encourage the rise and spread of antibiotic-resistant microbial strain in such an antibiotic-saturated environment (van den Bogaard & Stobberingh, 2000, Rawal *et al*., 2015).

Conclusion

Results from the study showed the widespread occurrence of antibiotic-resistant *E. coli* strains in poultry environments in the studied region. This has potential health implications. In addition to the potential of possible dissemination of pathogenic *E. coli* to the environment for instance through indiscriminate disposal of poultry waste. Contaminated poultry product can also serve as vehicle for the transfer of drug resistant *Escherichia coli* to humans. Moreso, drug resistant non-pathogenic *E. coli* also constitutes a hazard of transferring antibiotic resistant genes to pathogenic bacteria species. Therefore, there is need to regulate the indiscriminate use of antibiotics in poultry farming.

References

ADELOWO, O. O., FAGADE, O. E. & AGERSØ, Y. (2014). Antibiotic resistance and resistance genes in Escherichia coli from poultry farms, southwest Nigeria. *The Journal of Infection in Developing Countries,* **8,** 1103-1112.

ADENIPEKUN, E. O., JACKSON, C. R., OLUWADUN, A., IWALOKUN, B. A., FRYE, J. G., BARRETT, J. B., HIOTT, L. M. & WOODLEY, T. A. (2015). Prevalence and antimicrobial resistance in Escherichia coli from food animals in Lagos, Nigeria. *Microbial Drug Resistance,* **21,** 358-365.

AHMAD, M. & KHAN, A. U. (2019). Global economic impact of antibiotic resistance: A review. *Journal of global antimicrobial resistance,* 19**,** 313-316.

ALHAJI, N. B. & ISOLA, T. O. (2018). Antimicrobial usage by pastoralists in food animals in North-central Nigeria: The associated socio-cultural drivers for antimicrobials misuse and public health implications. *One Health,* s, antibiotics and antibiotic resistance. *FEMS microbiology reviews,* **41 (**6**)** 41-47.

BANIN, E., HUGHES, D. & KUIPERS, O. P. (2017). Bacterial pathogen 450-452.

BÉLANGER, L., GARENAUX, A., HAREL, J., BOULIANNE, M., NADEAU, E. & DOZOIS, C. M. (2011). Escherichia coli from animal reservoirs as a potential source of human extraintestinal pathogenic *E. coli*. *FEMS Immunology & Medical Microbiology,* 62**,** 1-10.

BENNANI, H., MATEUS, A., MAYS, N., EAST-MURE, E., STÄRK, K. D. & HÄSLER, B. (2020). Overview of evidence of antimicrobial use and antimicrobial resistance in the food chain. *Antibiotics,* **9** (49).

BISSONG, M. E. A., MOBEY, K. N., MUMA, V. & MKONG, P. B. (2023). Reduced susceptibility to carbapenems in Enterobacteriaceae and antimicrobial resistance profile of Escherichia coli strains isolated from clinical and zoonotic sources in the Bamenda Municipality, Cameroon. *The Pan African Medical Journal,* **44**.

FERENS, W. A. & HOVDE, C. J. (2011). Escherichia coli O157: H7: animal reservoir and sources of human infection. *Foodborne pathogens and disease,* **8,** 465-487.

HASSAN, M. M., AMIN, K. B., AHADUZZAMAN, M., ALAM, M., FARUK, M. & UDDIN, I. (2014). Antimicrobial resistance pattern against *E. coli* and Salmonella in layer poultry. *Res. J. Vet. Pract,* **2,** 30-35.

HOQUE, M., MOHIUDDIN, R. B., KHAN, M. M. H., HANNAN, A. & ALAM, M. J. (2019). Outbreak of Salmonella in poultry of Bangladesh and possible remedy. *J. Adv. Biotechnol. Exp. Ther,* **2,** 87-97.

JAMIU, M. O., GIWA, A., BELLO, I. K. & ABU SAEED, K. (2016). Prevalence and pattern of antibiotics use among residents of Ilorin metropolis in north central Nigeria.

JIBRIL, A. H., OKEKE, I. N., DALSGAARD, A. & OLSEN, J. E. (2021). Association between antimicrobial usage and resistance in Salmonella from poultry farms in Nigeria. *BMC Veterinary Research,* **17,** 234.

KHAN, S. A., IMTIAZ, M. A., SAYEED, M. A., SHAIKAT, A. H. & HASSAN, M. M. (2020). Antimicrobial resistance pattern in domestic animal-wildlife-environmental niche via the food chain to humans with a Bangladesh perspective; a systematic review. *BMC Veterinary Research,* **16,** 1-13.

MOFFO, F., MOUICHE, M. M. M., DJOMGANG, H. K., TOMBE, P., WADE, A., KOCHIVI, F. L., DONGMO, J. B., MBAH, C. K., MAPIEFOU, N. P. & NGOGANG, M. P. (2021). Poultry litter contamination by Escherichia coli resistant to critically important antimicrobials for human and animal use and risk for public health in cameroon. *Antibiotics,* **10,** 402.

MONDAL, T., DEY, S., ISORE, D. P., SAMANTA, S., BANERJEE, A., SINGH, A. D., SHARMILA, L., HAJARIKA, Z. H. & BATABYAL, K. (2023). Detection of drug-resistant extended-spectrum and AmpCβ-lactamases producing Escherichia coli from poultry faecal samples in West Bengal, India. *Explor Anim Med Res,* **13,** 31-38.

MORA, A., VISO, S., LÓPEZ, C., ALONSO, M. P., GARCÍA-GARROTE, F., DABHI, G., MAMANI, R., HERRERA, A., MARZOA, J. & BLANCO, M. (2013). Poultry as reservoir for extraintestinal pathogenic Escherichia coli O45: K1: H7-B2-ST95 in humans. *Veterinary microbiology,* **167,** 506-512.

NOPPON, B., SAMPAO, S. W., SORNPLANG, P., SOPA, A., SUKON, P. & KANISTANON, K. (2018). Multi-drug resistance characteristics of *E. coli* isolated from water sources in chicken farms, Khon Kaen, Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health,* 49**,** 1034-1042.

OKOLI, I., NDUJIHE, G. & OGBUEWU, I. (2006). Frequency Of Isolation Of Salmonella From Commercial Poultry Feeds And Their Anti-Microbial Resistance Profiles, Imo State. *Nigeria Online J Health Allied Scs,* **2**, 3.

OLATOYE, I. O. & BASIRU, A. (2013). Antibiotic usage and oxytetracycline residue in African catfish (Clarias gariepinus) in Ibadan, Nigeria. *World Journal of Fish and Marine Sciences,* 5**,** 302-309.

PANCHAL, P. P., PATEL, J. M., VIHOL, P. D., RAVAL, J., PATEL, D., DANGER, N., KALYANI, I. & PATEL, N. (2020). Serotyping

and Prevalence of Escherichia coli Infection in Poultry of South Gujarat based on Culture Isolation and Identification. *Int. J. Curr. Microbiol. App. Sci,* **9,** 3876-3883.

PUVAČA, N. & DE LLANOS FRUTOS, R. (2021). Antimicrobial resistance in escherichia coli strains isolated from humans and Pet animals. *Antibiotics,* 10**,** 69.

RAHMAN, M. M., HUSNA, A., ELSHABRAWY, H. A., ALAM, J., RUNA, N. Y., BADRUZ-ZAMAN, A., BANU, N. A., AL MAMUN, M., PAUL, B. & DAS, S. (2020). Isolation and molecular characterization of multidrug-resistant Escherichia coli from chicken meat. *Scientific Reports,* **10,** 21999.

RAVI, A., AVERSHINA, E., LUDVIGSEN, J., L'ABÉE-LUND, T. M. & RUDI, K. (2014). Integrons in the intestinal microbiota as reservoirs for transmission of antibiotic resistance genes. *Pathogens,* **3,** 238-248.

RAWAL, I., JOSHI, H. & CHAUDHARY, B. (2015). Study of antibiotic resistance pattern of Escherichia coli PS 58 isolated from Pichhola Lake of Udaipur, Rajasthan, India. *Research Journal of Recent Sciences ISSN,* 2277**,** 2502.

SARBA, E. J., KELBESA, K. A., BAYU, M. D., GEBREMEDHIN, E. Z., BORENA, B. M. & TESHALE, A. (2019). Identification and antimicrobial susceptibility profile of Escherichia coli isolated from backyard chicken in and around ambo, Central Ethiopia. *BMC veterinary research,* **15,** 1-8.

SOMMER, M. O., MUNCK, C., TOFT-KEHLER, R. V. & ANDERSSON, D. I. (2017). Prediction of antibiotic resistance: time for a new preclinical paradigm? *Nature Reviews Microbiology,* **15,** 689-696.

STROMBERG, Z. R., JOHNSON, J. R., FAIRBROTHER, J. M., KILBOURNE, J., VAN GOOR, A., CURTISS 3RD, R. & MELLATA, M. (2017). Evaluation of Escherichia coli isolates from healthy chickens to determine their potential risk to poultry and human health. *PloS one,* 12**,** e0180599.

TACCONELLI, E., SIFAKIS, F., HARBARTH, S., SCHRIJVER, R., VAN MOURIK, M., VOSS, A., SHARLAND, M., RAJENDRAN, N. B., RODRÍGUEZ-BAÑO, J. & BIELICKI, J. (2018). Surveillance for control of antimicrobial resistance. *The Lancet Infectious Diseases,* 18**,** e99-e106.

VAN DEN BOGAARD, A. E. & STOBBERINGH, E. E. (2000). Epidemiology of resistance to antibiotics: links between animals and humans. *International journal of antimicrobial agents,* **14,** 327-335.

YASMIN, S., KARIM, A.-M., LEE, S.-H. & ZAHRA, R. (2022). Temporal Variation of Meropenem Resistance in *E. coli* Isolated from Sewage Water in Islamabad, Pakistan. *Antibiotics,* **11,** 635.