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🌐 isolation of Salmonella and pathogenic E. coli in broiler farms in Uganda

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Disclaimer

N/A

Abstract

This study investigated the prevalence, antimicrobial resistance, and extended-spectrum β-lactamase (ESBL) production in *Escherichia coli* and *Salmonella* isolated from broiler farms in Wakiso district, Uganda. A cross-sectional design was employed between August and September 2021. A total of 216 farms were selected using simple random sampling, and pooled samples of fresh fecal droppings and cloacal swabs were collected. Microbiological isolation and identification were conducted using on selective media, followed by phenotypic and genotypic assays to determine antimicrobial resistance profiles.

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method, and results were interpreted according to CLSI 2020 guidelines. Phenotypic ESBL production was screened using the double-disc synergy method. DNA extraction and conventional PCR were employed to confirm the presence of the *blaTEM* resistance gene. Sdata was analysed using Stata to identify significant associations between variables.
The findings highlight a significant prevalence of antimicrobial resistance and the emergence of ESBL-producing *Escherichia coli* and *Salmonella* in broiler farms. This calls for antimicrobial stewardship in poultry production.

Attachments

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E.coli$ Salmonella p...
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Image Attribution

N/A

Guidelines

N/A

Materials

This study was conducted in Wakiso district a metropolitan district in the central region of Uganda that partly encircles Kampala district. The district coordinates are 00 24N, 32 29E. With a projected population of 2,915,200 million, Wakiso district is still Uganda's most populous Higher Local Government (HLG) (1). The population is projected to grow at 4.1% annually, which increases the demand for food, particularly poultry productsThe district was regarded as the top producer of poultry, producing 7.4% of all the chickens in the country (2).

Study design

This was a cross-sectional study focused on broiler farms from August to September 2021 based on the Food and Agriculture organization (FAO) categorization sector level 3 within selected counties in Wakiso district.

Sample Size Determination

The sample size was determined using Kishi-Leslie (1965) formula using 83% prevalence (3).

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

n = study sample size required
Z= critical value associated with 95% confidence interval = 1.96
P = Estimated prevalence of 83%of *E coli* and salmonella d = margin of error = 0.05

$$n= \frac{1.96^2 (0.83 \times 0.17)}{0.05^2}$$

n= 216 samples
Therefore, 216 farms within Wakiso District were considered. The farm managers of the farms were interviewed to understand the associated factors that predispose these pathogens with consent.

Sample Collection

Simple random sampling to select the sub-counties was employed while purposive sampling used the information provided by the district veterinary office to

identify the villages.   For the selected and eligible farms
three swabs from fresh fecal droppings and two swabs from the cloaca were
collected from randomly selected old (5-7weeks) birds (4). Five swabs from one farm on different
chickens were considered to represent one sample (Pooling of samples).   The swabs were placed into a plain labeled
tube containing Amie's transport media without charcoal. This was then put into
a cool box and transported to the laboratory within 24 hours.

Isolation and identification of microorganisms

The pooled
samples were aseptically mixed into 9 ml of autoclaved Buffered Peptone Water
(BPW) (Hi Media M1494, Mubi, India) in a sterile 50ml falcon tube with a lid to
generate a pre-enriched sample and incubated for 16-24 hours at 37°C
aerobically.

The
enriched sample was picked and inoculated onto a Chromagar^{TM} of
Salmonella designed for the
presumptive identification, qualitative direct detection, and differentiation
of *Salmonella* species (5). Plates were incubated at 37^{0}C
for 24 hours. Presumptive colonies for *Salmonella*
(mauve-pink, raised, and smooth colonies) were selected for identification.
For
isolation of pathogenic *E.coli*, the
pre-enriched sample was inoculated on Sorbitol MacConkey agar plates and
incubated at 37 °C for 24 hours. The colonies of pathogenic *E. coli* were smooth, raised, had entire
margins, and were colorless on SMAC.
The
presumptive organisms were subcultured on nutrient agar and identified through
gram staining, Triple Ion Sugar (TSI) test, the IMViC test, Methyl Red, Voges-
Proskauer, Sulphur indole motility, and Simmons citrate utilization (figure 2).
All reagents used were from (Oxoid, England) (6).

Bacterial antibiotic susceptibility testing

Bacterial
suspension was adjusted to 0.5McFarand standard and inoculated on Muller Hinton
agar medium (Oxoid CM0337 Basingstoke, England) using
surface spreading method. The antibiotic discs listed in Table 1 (all from
Oxoid, England) were used. The plates were incubated for 24 hours at 37°C. The
results were read and interpreted according to Clinical Laboratory Standards

Institute, 2020 (7). *E. coli* ATCC 25922 (American Type Culture Collection, Rockville, MD, USA) was used as a reference control strain.

Table 1: *Commonly used antibiotics at NaLIRRI, 2022 obtained from Oxoid Ltd suppliers, and the discs were selected based on CLSI (28th Edition, 2020) and their availability at the NALIRRI (National Livestock Resources Research Institute) Microbiology laboratory.*

Antibiotic Class	Antibiotics used for both E. coli and Salmonella isolates
Aminoglycosides	Gentamicin (GM) (10 μg/ml)
Carbapenem	Meropenem (MEM) (10 μg/ml)
Cephalosporins class III	Ceftriaxone (CRO) (30 μg/ml)
Amphenicol	Chloramphenicol (CHL) (30 μg/ml)
Cephalosporins class IV	Cefepime (CPM) (30 μg/ml), Cefotaxime (CTX) (30μg/ml), Ceftazidime (CTZ) (30μg/ml)
Quinolones	Ciprofloxacin (CIP) (5 μg/ml)
Macrolides	Erythromycin (EM) (30 μg/ml)
Penicillin	Ampicillin (AMP) (10 μg/ml)
Tetracyclines	Tetracycline (OXT) (30 μg/ml)
Sulphonamides/ Trimethoprim	Co-trimoxazole (CXT) (25 μg/ml)

Phenotypic Screening of Enterobacteriaceae for Extended-spectrum β-lactamases (ESBLs) production

Cefotaxime *(30µg)* and Ceftazidime *(30µg)* antibiotic discs were used to phenotypically test Enterobacteriaceae for the development of ESBLs and incubated overnight at 37 ^{0}C on Muller Hinton agar (7).

Double Disc Synergy method

A sterile cotton swab was used to surface spread the bacterial suspension onto a Mueller Hinton agar plate. The antibiotic discs used were Ceftazidime (CAZ) (30µg) alone and Ceftazidime in combination with Clavulanic acid (CAL) (30/10µg) (8). The discs were spaced approximately 30 mm apart. The plates were incubated at 37° C overnight. Both the single disc and the combined disc's zones of inhibition were measured. The results were interpreted according to CLSI guidelines (8).

Genomic DNA extraction

Bacterial genomic DNA extraction was conducted on all 18 *Salmonella* and 57 Pathogenic *E.coli* following manufacturer instructions of the Bio line ISOLATE II genomic DNA kit (Cat No. Bio-52065 Lot No. IS502-B054750). The presence of genes encoding for ESBL (*bla*TEM gene) was detected using conventional PCR amplification using primers listed in (table 2).

Table 2: Primers used in the PCR reaction.

*bla*TEM	(F) TGG GTG CAC GAG TGG GTT AC	526	58	Tenover et al., 1994
(R) TTA TCC GCC TCC ATC CAG TC				

The PCR master Mix reagents were prepared by mixing 12.5 µL master mix consisting of One Taq quick load two times master mix/w standard buffer, dNTPs and Taq polymerase (M0486S), 1.5 µL forward (100 µM), 1.5 µL primary reverse (one hundred µM), and5 µL DNA template and RNAse-free dH2O up to 25 µL.

The PCR process was carried out in a thermocycler (Perkin Elmer, Wellesley, MA, USA) with a pre-denaturation cycle of 95°C for 15 min, followed by a DNA amplification stage with 30 cycles (94°C for 1 min, 58°C for 1 min, and 72°C for 1 min) and final extension cycle of 72°C for 5 min.

DNA Amplicons were electrophoresed using 1.5% agarose gel, in Tris-Borate EDTA buffer (TBE) 1×concentration, Safe View Classic^{TM} DNA stain, 6x loading dye (Thermo Scientific), and DNA ladder/marker 100 bp (Sigma-Aldrich, Inc., Saint Louis, MI, USA)DNA Bands were visualized on a Dark reader Transilluminator.

Data Management and Analysis

All records of the analysis were recorded in the lab register as a hard copy backup. Samples were assigned codes and Excel spreadsheets were used to enter the raw data, which were then exported to Stata (Version 12, Special Edition, College Station, Texas USA) for analysis. Double entry of data was done to rule out any errors. Frequency tables and graphs were used to present descriptive statistics.

The 95% level of significance where the Pearson value ($p < 0.05$) determined the variables of statistical significance of Quantitative data.

Ethical Consideration

Approval was obtained from Mbarara University of Science and Technology; the Institutional Ethical Review Committee (MUST-2021-141), and at the ministry level, the permanent secretary Ministry of Agriculture, Animal Industries, and Fisheries, the district's chief administrative officer, and the district veterinarian.

Before engaging the farms, we sought clearance from the farm owners to access and collect samples from their farms. Data and bacterial isolates obtained for this investigation was handled with confidentiality.

Troubleshooting

Safety warnings

❗ N/A

Ethics statement

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Before start

The emergence and re-emergence of zoonotic bacterial infections and the upsurge reflected in current trends of antimicrobial-resistant bacteria is a major global concern. *Salmonella* spp and *Escherichia coli (E. coli)* are the two most important food-borne pathogens of public health interest incriminated in poultry products worldwide hence necessitating constant monitoring of microbial food safety measures.

Prevalence and antimicrobial resistance of Salmonella and pathogenic E. coli in broiler farms, Wakiso district, Uganda

1   📄 E.coli$ Salmonella protocol.docx 26KB

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