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Prevalence and Antimicrobial Susceptibility of Escherichia Coli and Salmonella Isolates from Feeds, Litter and Cloacal Swabs from Broiler Chicken in Kalerwe and Kasubi Markets

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ABSTRACT

The demand for poultry products has increased in the recent past especially as more people realize the nutritional and economic value of chicken and their products. Consequently, many poultry farms have sprung up particularly in and around urban centres. However high mortalities in chicken and reduced individual sizes that are attributed to bacterial infections have been reported. The present study investigated the prevalence and antimicrobial susceptibility of E. coli and Salmonella isolates from the feed, litter and cloacal swab samples of broiler chicken sold in Kalerwe and Kasubi markets from January 2019 to May 2019.

A total of 180 samples were collected from Kalerwe and Kasubi markets where 90 samples(30 each of litter, feed and cloacal swabs) were from each of the markets. Using standard bacteriological techniques, E. coli and Salmonella were isolated from 126(70%) and 3(1.67%) samples respectively. The overall prevalence of E. coli and Salmonella in Kasubi market was 84.4% and 2.2% respectively and that in Kalerwe market was 55.6% and 1.1% respectively. The overall prevalence of E. coli in feed, litter and cloacal swab samples was 51.7%, 70% & 88.3% respectively and that of Salmonella was 0%, 1.7% & 3.3% respectively.

Furthermore, isolates were subjected to antimicrobial susceptibility test by disc diffusion method using six commonly used antibiotics in both human and veterinary practice. The study revealed that E. coli isolates were more sensitive to gentamicin(83.4%) and ciprofloxacin(64.5%) whereas most resistance was to tetracycline(73.8%), ampicillin(70.6%), chloramphenicol(66.7%) and nalidixic acid(56.3%). All Salmonella isolates showed resistance to nalidixic acid, ciprofloxacin and chloramphenicol.

This study showed a high resistance rate against the antibiotics commonly used in poultry and humans. These findings confirm a significant increase in the incidence of antimicrobial resistance in E. coli and Salmonella which is most likely due to using of antibiotics as feed additives for growth promotion and inappropriate use of antibiotics for prevention and treatment of poultry diseases. Furthermore, the results suggest that poultry litter and feed are major sources of E. coli contamination to birds.

INTRODUCTION

Background

The world has over 23 billion poultry birds about three per person on the planet and about 5 times more than 50 years ago. They are kept and raised in a wide range of production systems to provide mainly meat, eggs and manure for crop fertilization. The specialized type of chicken reared for meat are broiler chicken. Broiler production is one of the fastest-growing agricultural sub-sector. Poultry meat is among the most common animal sources of food consumed at the global level through a wide diversity of cultures, traditions, and religion making it key to food security and nutrition. The biggest poultry meat producers are the United States, with almost 20 million tons a year, followed by China, with 18 million tons, the EU and Brazil with about 13 million tons(Mottet & Tempio, 2017).

In Uganda, the demand for poultry products has increased in the recent past especially as more people realize the nutritional and economic value of chicken and their products. Consequently, many poultry farms have sprung up particularly in and around urban centres(Waiswa, 2006). Unfortunately, many farmers are complaining about high economic losses ranging from severe broiler chicken mortality to reduced individual sizes. Among other challenges encountered in broiler production, are bacterial infections that cause economic losses. The most common bacterial infections are caused by Salmonella, E. coli, Campylobacter and Staphylococcus aureus among others.

Broilers get exposed to the potentially pathogenic bacteria through feeding on contaminated feeds and drinking water. Others being transport equipment and environmental factors such as air, litter, unclean facilities and vectors such as insects, humans and rodents. Because of these potential bacteria sources, broiler production has been challenged with many mortalities that have led to frequent antibiotics to increase productivity on the farm.

In veterinary practice, antibiotics are used in food-producing animals more especially on poultry farms for treatment of bacterial infections, prophylaxis and also as growth promoters in animal feeds. Tetracyclines and penicillin are the most commonly used, although several others such as fluoroquinolones are also used(Uganda Nation Academy of Science, 2015). Furthermore, poultry farmers practice empirical treatment of poultry diseases without seeking veterinary assistance. This continued misuse and overuse of antibiotics in production is considered the most important factor promoting the emergence of antibiotic-resistant commensals, pathogenic and zoonotic bacteria(Persoons et al., 2012). These pathogens are disseminated to the surrounding environment thus easily transmitted to humans. For example, identical E. coli antibiotic-resistant plasmids have been isolated from broilers and human origin(Leverstein et al., 2011).

Antimicrobial resistance has been and is still a global challenge of the 21st century. This has threatened the effective prevention and treatment of the ever-increasing range of infections caused mostly by bacteria, parasites, viruses and fungi(Prestinaci, Pezzotti, & Pantosti, 2015). Today antibiotic resistance has spread to a point that the general population is at risk. For example, because of multidrug-resistance in non-typhoidal Salmonella, treatment with first-line drugs is often no longer an alternative, and this puts pressure on the use of second or third line drugs which are relatively expensive(Odoch et al., 2017).

Unfortunately, antibiotics are used without proper evaluation of the need to use them. Antibiotics are normally put in broiler feeds and drinking water which in most cases might not be necessary. The overuse and misuse of antibiotics in poultry farms could be the reason behind the emergence of antibiotic-resistant bacteria causing infections in humans. Humans get exposed to these pathogens through ingesting contaminated poultry products. The environment around poultry houses and farms is at a high risk of having the antibiotic-resistant pathogens. This is through the deposition of poultry litter to the nearby environment. The research aims are to determine the prevalence of E.coli and Salmonella in the poultry feeds, litter and cloacal swabs, and determine their susceptibility patterns to the commonly used antibiotics.

Problem Statement

In Uganda, broiler production is one of the growing sectors since most of the people have engaged themselves in this profitable business. However, this has not been the case to some extent due to high mortalities in chicken and reduced individual sizes. These economic losses are mainly attributed to bacterial infections caused by pathogens like Salmonella and E. coli. Sources of these pathogens could be the environment in which these broiler chicken are raised. To overcome these challenges, antibiotics are used in poultry as in humans for therapy, control of bacterial infections and growth promotion. Antibiotics are administered to whole flocks rather than individual birds. In this way, antibiotic use is abused which has led to the emergence of antibiotic-resistant pathogens which cause infections that cannot be treated thus more economic losses due to high broiler mortalities on the farms. The antibiotic-resistant pathogens can be recovered in litter and fecal matter thus posing a big challenge to the community as the resistant genes can be disseminated to other bacteria. However little data has been documented on the prevalence and antibiotic susceptibility patterns of pathogens like Salmonella and E. coli of broiler origin in Kampala.

General Objective

To determine the prevalence and antimicrobial susceptibility of E. Coli and Salmonella isolates from poultry feeds, litter and cloacal swabs of broiler chicken in kalerwe and kasubi markets.

Specific Objectives

To determine the prevalence of E. coli and Salmonella isolated from broiler feeds, litter and cloacal swabs of broiler chicken sold in Kalerwe and Kasubi markets.

To determine the susceptibility patterns of Salmonella isolated from broiler feeds, litter, cloacal swabs of broiler chicken sold in Kalerwe and Kasubi markets.

To determine the susceptibility patterns of E. Coli isolated from broiler feeds, litter and cloacal swabs of broiler chicken sold in Kalerwe and Kasubi markets.

Research Question

What is the prevalence of E. coli and Salmonella isolated from broiler feeds, litter and cloacal swabs of broiler chicken sold in Kalerwe and Kasubi markets?

What are the antibiotic susceptibility patterns of E. coli isolated from broiler feeds, litter and cloacal swabs of broiler chicken sold in Kalerwe and Kasubi markets?

What are the antibiotic susceptibility patterns of Salmonella isolated from broiler feeds, litter and cloacal swabs of broiler chicken sold in Kalerwe and Kasubi markets?

Justification

In Uganda, there is a lack of a regulatory environment that ensures only products of acceptable standards are offered to poultry farmers. In particular, small scale farmers are vulnerable in this respect to substantial feeds and veterinary drugs which are traded openly without much control across the country. Poultry production in Uganda is hence accompanied by intense antibiotic use. This is mainly to boost production through prophylaxis and growth promotion by antibiotics added in feeds and drinking water of chicken. Therefore isolating E. coli and Salmonella more especially from feeds and litter will create more awareness about the possible sources pathogens to chicken hence reinforcing interventions required to reduce their effects. Furthermore, investigating resistance in Salmonella and E. coli is of importance in understanding antibiotic resistance of these bacteria that could be linked to the negligent use of antibiotics in production. This will also aid in identifying possible sources of resistant pathogens causing infections in human

beings especially E. coli which occupies multiple niches in both humans and other animal hosts.

In particular, little data has been documented on the prevalence and antibiotic resistance profiles of Salmonella and E. coli of broiler origin in Kampala markets. Hence there is an urgent need for the information obtained from the study.

Significance

The information generated from the study will create awareness to concerned stakeholders about the emergence of antibiotic-resistant pathogens from poultry. This will act as a basis for the design of suitable interventions to minimize the emergence of antibiotic-resistant bacteria. Hence stringent control over the use of antibiotics in poultry production.

LITERATURE REVIEW

Classification of Salmonella

Salmonella is a genus that belongs to family Enterobacteriaceae. The two species of Salmonella are Salmonella enterica and Salmonella bongori. Salmonella bongori is restricted to cold-blooded animals, particularly reptiles. Salmonella enterica is further divided into six subspecies that includes over 2,600 serotypes (Gal-Mor, Boyle, & Grassl, 2014). Salmonella enterica subspecies are found worldwide in all warm-blooded animals and the environment. Salmonella serotypes are divided into two main groups, typhoidal and Non-typhoidal serotypes. Non-typhoidal serotypes are more common and usually cause self-limiting gastrointestinal disease. They affect a range of animals and are zoonotic, meaning they can be transferred between humans and other animals. Typhoidal serotypes include Salmonella typhi and Salmonella paratyphi A, which is adapted to humans and doesn't occur in other animals.

Diagnostic and Phenotypic Characteristics of Salmonella

Salmonella is a rod-shaped, Gram-negative facultative anaerobe that belongs to the family Enterobacteriaceae (Barlow & Hall, 2002). Salmonella species are non-spore forming, predominately motile enterobacteria with peritrichous flagella. They are facultative aerobes and chemotrophs obtaining their energy from oxidation and reduction reactions with organic sources.

Prevalence of Salmonella

Salmonella is frequently found in poultry and represents an important source of human gastrointestinal infections. The prevalence of Salmonella isolated from the caeca of Ecuadorian broilers at slaughter age was reported to be 16% (Vinueza-Burgos et al., 2016). However, according to the study done in the farming communities of northern Thailand, the prevalence of Salmonella in chicken cloacal swabs was found to be 1% (Hanson, Kaneene, Padungtod, Hirokawa, & Zeno, 2002). And in a study on broiler farms in the Dinajpur district of Bangladesh, the overall prevalence of Salmonella was reported to be 49.91% with the prevalence in feed and litter as 29.16% and 66.66% respectively (Islam, Islam, & Fakhruzzaman, 2014).

Pathogenesis and Virulence

Salmonella serotypes are capable of causing infections in both humans and poultry (Steve Yan et al., 2004). Salmonellosis is a major bacterial infection caused by several Salmonella serotypes. It is associated with several mortalities and economic losses in broiler production (Haider et al., 2009). Chickens can be infected with many different serovars of Salmonella; some serovars such as S. Pullorum and S. Gallinarum are host-specific for chickens, whereas other serovars such as S. Typhimurium, S. Enteritidis and S. Heidelberg are capable to infect a wide range of hosts.

The pathogenicity of Salmonella depends on its ability to invade, multiply and survive in the cells. Salmonella bacteria invade through the oral route. Once they enter the lumen of the intestines, they multiply and some attach on the microvilli of the mucosa by adhesion (Humbert & Salvat, 2010). Attachment is followed by degeneration of the microvilli to form breaches in the cell membrane through which Salmonella enters the cell and further multiplies. Bacteria further invades the cecal tonsils and the Peyer's patches from where they engulfed by macrophages. Macrophages transport through the bloodstream and/or the lymphatic system to other organs in the body such as the liver and spleen. It's in these organs where the bacteria further multiplies (Barrow, Huggins, & Lovell, 2014).

The major virulence factor displayed by Salmonella is endotoxin production where the local response is enteritis and gastrointestinal disorders (Iushchuk & Tendetnik, 2013).

Clinical Manifestation

Based on the annual reports from various district Veterinary offices, pullorum disease and fowl typhoid are among the most prevalent poultry diseases. Pullorum disease and fowl typhoid or septicaemic diseases are infectious, acute or chronic bacterial diseases caused by Salmonella pullorum and Salmonella gallinarum respectively (Lutful Kabir, 2010). The causative agents can be transmitted primarily through eggs or other means such as mechanical transmission, carrier birds (apparently healthy birds which shed organisms) and contaminated premises (Berchieri et al., 2008). The bacteria invade through the respiratory tract and digestive system. Pullorum disease is highly fatal to chicks which die so soon after hatching and no signs are observed. Survivors are usually stunted and unthrifty. Infections in young birds may be indicated by droopiness, ruffled feathers, a chilled appearance with birds huddled around the source of heat, white diarrhea with the pasting of the vent feathers and labored breathing.

Fowl typhoid occurs in young adults. It is associated with sudden or sporadic mortality, listless-ness, green or yellow diarrhea with pasting on the vent feathers, loss of appetite, increased thirst and a pale anemic appearance of combs and wattles.

In humans, Salmonella of poultry origin causes majorly Non-typhoidal salmonellosis. It is the major zoonotic disease that can be contracted through contact with infected animals and their products (Arya et al., 2017). Fecally contaminated foodstuffs like meat (poultry meat), eggs, dairy products and sometimes

vegetables are the major sources of salmonellosis in humans (Odoch et al., 2017). In all forms of infection, organisms enter via the oral route and may produce either clinical or sub-clinical infections. Salmonella of poultry origin may cause either Septicemia or Gastroenteritis in humans (Hohmann, 2011).

Gastroenteritis is mainly due to food poisoning as a result of contamination of food with mainly S. typhimirium and S. enteritidis among others. It is normally associated with vomiting, abdominal cramps and diarrhea which occur after incubation of 1-3days (Mumy, 2014). This is suggestive of the ingestion of a large number of organisms that liberate toxins which result in local violent irritation of the mucous membranes. However, there is no invasion of the bloodstream or distribution to the organs which is the case in septicemia. In septicemia, the organisms invade the blood following ingestion through the oral route (Eng et al., 2015). The organisms are widely disseminated and tend to cause focal suppuration, pneumonia, abscesses, menegitidis and osteomyelitis.

Microbiology of E.coli

Overview of E. coli

Escherichia coli is a Gramnegative, facultative anaerobe bacte rium of the Enterobacteriaceae family (Nhung, Chansiripornchai, & Carrique-Mas, 2017). The primary and secondary habitats of E. coli are the intestinal tract of warm-blooded animals and the environment. Although many E. coli strains are harmless commensals, a subset that has acquired the ability to cause intestinal and extraintestinal diseases (Stromberg et al., 2017). The harmless strains are part of the normal flora of the gut and can benefit their hosts by producing vitamin K2, and preventing the colonization of the intestine with pathogenic bacteria, having a symbiotic relationship. E. coli is expelled into the environment within fecal matter. The bacterium grows massively in the fresh fecal matter under aerobic conditions for 3 days, but its numbers decline slowly afterward.

E. coli and other facultative anaerobes constitute about 0.1% of gut flora. Fecal–oral transmission is the major route through which pathogenic strains of the bacterium cause disease. The bacterium can be cultured easily and inexpensively in a laboratory setting and has been intensively investigated for over 60 years. E. coli is chemoheterotrophic whose chemically defined medium must include a source of carbon and energy. E. coli is the most widely studied prokaryotic model organism, and an important species in the fields of biotechnology and microbiology, where it has served as the host organism for the majority of work with recombinant DNA.

Prevalence of E. coli

Like salmonella, E. coli is among the leading pathogens causing bacterial infections in broilers. This pathogen is almost always isolated from the environment in which broilers are raised. In a study done on broiler farms of Dinjpur in Bangladesh, the overall prevalence of E. coli was reported to be 62.5% with the prevalence in feeds and litter as 37.5% and 87.5% respectively (Islam et al., 2014).

E. coli Pathogenesis and Virulence

Most E. coli strains don't cause disease but virulent strains cause various diseases in both animals and human beings. In poultry as in humans, E. coli resides in the lower digestive tract which it colonizes in the first 24hrs after hatching or birth (Ballou et al., 2016). Although many E. coli are harmless, some have acquired the ability to cause intestinal and extraintestinal diseases.

Extraintestinal pathogenic E. coli (ExPEC) strains cause diverse infections outside the intestinal tract of humans and animals (Mellata, 2013a). Based on host and site ExPEC are sub-classified as neonatal meningitis E. coli (NMEC), Sepsis associated E. coli(SEPEC), Uropathogenic E. coli (UPEC), which cause new-born meningitis, sepsis and urinary tract infections(UTI), respectively; and Avian pathogenic E. coli (APEC) which mainly causes respiratory and systemic disease in poultry (Stromberg et al., 2017).

Although E. coli is present in the normal microflora of the intestinal tract and other host mucosal surfaces other strains possessing specific virulence attributes designated as APEC can cause disease (Dho-moulin et al., 2016). APEC is mostly associated with respiratory tract or systemic infections that lead to a variety of diseases that are responsible for severe economic losses. APEC are the causative agents of colibacillosis which is known to cause heavy economic losses in poultry (Kabir, Kabir, & Lutful, 2010).

A variety of virulence factors have been implicated in promoting these extraintestinal diseases in avian species; adhesions, iron acquisition systems and hemolysins (Cooke & Ewins, 2004). Antibacteriacidal factors(outer membrane A, protein for increased serum survival, lipopolysaccharide, K-1 capsule and colicin production) and toxins (Silver et al., 2006).

Diagnosis of E. coli and Salmonella Infections in Poultry

The dominant infections caused by E. coli and Salmonella are colibacillosis and salmonellosis respectively. Typically infections of E. coli are based on clinical features and typical macroscopic lesions in colibacillosis. The diagnosis is majorly obtained by E. coli isolation from the affected tissues like cardiac blood liver, spleen, and bone marrow in birds that have been presented with clinical symptoms. In acute cases, isolation is possible at 6hrs to 3 days after infection whereas in sub-acute cases isolation is possible at 7days after infection(Kabir et al., 2010). Selective media such as Mac Conkey and Eosin methylene blue are used for isolation. And further identification is based on biochemical tests (Indole, fermentation of glucose with gas production, inability to use citrate as a carbon source, absence of hydrogen sulphite production and urease).

Diagnosis of avian salmonellosis is majorly based on isolation, identification and serotyping of Salmonella strains. However, infection in mature birds can be based on serological tests, followed by necropsy and evaluation complemented by microbial culture, biochemical tests and maybe typing for confirmation. For increased sensitivity, PCR is used in the diagnosis of both salmonella and E. coli (Bayardelle & Zafarullah, 2012).

Prevention of Salmonella and E. coli Infections in Broilers

Prevention of fecal egg contamination at the point of egg-laying is possible by fumigating with two hours after laying. Removal of cracked fecally contaminated eggs is the best way to prevent the hatching of infected birds (Lutful Kabir, 2010).

In chicks, contamination with APEC and Salmonella from the environment can be reduced through competitive exclusion. This is avoiding colonization of the intestinal tract with pathogens (salmonella and E. coli) by inoculating day one chicks with normal bacterial flora from adult healthy broilers.

Good hygiene and proper management practices are key prevention strategies of these infections. Broilers should be raised in a clean and sanitized environment whose humidity and ventilation are optimal. Mechanical transmission of pathogens from equipment, footwear, human clothing and crates should be avoided by adequate cleaning of those materials. Proper housing infrastructure is important to avoid stress factors such as overcrowding that predispose the birds to bacterial infections (Lutful Kabir, 2010).

Prevention has also been achieved through vaccination of birds against the causative agents. However, this has been a challenge due to the availability of many strains of the pathogens that cause these infections. Therefore the vaccines available don't protect the birds from that wide of bacterial strains. Water and feeds given to the birds should be free from bacteria more especially salmonella.

Antimicrobial Susceptibility

Antimicrobial susceptibility tests are tests done to determine which specific antibiotics a particular bacteria or fungus is sensitive to. The performance of antimicrobial susceptibility testing by the clinical microbiology laboratory is important to confirm susceptibility to chosen empirical antimicrobial agents or to detect resistance in individual bacterial isolates (Jorgensen & Ferraro, 2009). The most common methods for in vitro antimicrobial susceptibility testing are; Broth dilution tests, Disk diffusion tests and automated instrument systems.

Disk Diffusion Test

It is a simple and practical method that has been well standardized. The test is performed by applying a bacterial inoculum of approximately 1.5 x 108 CFU/ml (a suspension that matches that of 0.5 McFarland standard) to the surface of a large (150mm) Mueller Hinton agar plate using a sterile wire loop. Up to 12 commercially prepared, fixed concentration, paper antibiotic disks are prepared on the inoculated agar surface. The plates are incubated for 16-24hrs at 35oc before the determination of results. The zones of growth inhibition around each of the antibiotic disks are measured to the nearest millimetre. The diameter of zones is related to the susceptibility of the isolate and the diffusion rate of the drug through the agar medium. The zone diameters of each drug are interpreted using set criteria, for example, the criteria published by the Clinical and laboratory standards institute. The results obtained are qualitative in the category of susceptibility (that is susceptible, intermediate or resistant) (Balouiri, Sadiki, & Ibnsouda, 2016).

Disk diffusion test is advantageous in a way that it is simple as it doesn't require special equipment, provision of easily interpreted categorical results and flexibility in the selection of antibiotic disks for testing. However, it is disadvantageous in the way that there is a lack of mechanization or automation for the test and also not all fastidious or slowly growing bacteria can accurately be tested by this test.

Antimicrobial Susceptibility of Salmonella

Salmonella has rapidly gained resistance to antibiotics most commonly used in the veterinary sector and also the human sector for the treatment of Salmonella infections. The most commonly used antibiotics are tetracycline, sulphonamides, ciprofloxacin, ampicillin and macrolides. In a study done on Portuguese poultry products revealed that 75% of the Salmonella isolates were resistant to one or more antimicrobial agents (Antunes, Réu, Sousa, Peixe, & Pestana, 2003).

Antimicrobial Susceptibility of E. coli

Antibiotic-resistant E. coli have been recovered in previous studies performed in broilers. In particular Avian pathogenic E. coli are often resistant to antibiotics used in poultry including tetracycline, chloramphenicol, sulphonamides, aminoglycosides and β-lactamases (MA Rahman, Samad, Rahman, & Kabir, 2009). In a study performed on chicken diagnosed with colibacillosis, the E. coli isolates obtained showed increased resistance to antibiotics of human and veterinary therapeutic significance. The highest rates of resistance were to trimethoprim-sulfamethoxazole (100%), oxytetracycline (100%), ampicillin (83%), enrofloxacin (83%) and chloramphenicol (81%)(Li et al., 2007). Amazingly there has been an increase in the prevalence of these antibiotic-resistant pathogens. More especially the Extended beta-lactamase-producing E. coli has been recovered in the gastrointestinal tract of healthy broilers(Leverstein-van Hall et al., 2011).

Mode of Action of Antibiotics

Antibiotics are selective toxins that inhibit enzymes that are either unique to prokaryotic cells or sufficiently different so that toxicity to mammalian hosts is low(McDermott, Walker, & White, 2003). Antibiotics used, fall in one of the four major mechanisms of actions, these include inhibitors of bacterial cell wall synthesis, protein synthesis, nucleic acid synthesis or disrupting the cell membrane integrity.

Tetracyclines

Tetracyclines are protein synthesis inhibitors. This is achieved by binding on the 16s part of the 30s ribosomal subunit thus preventing the aminoacyl tRNA from binding to the A site of the ribosome (Brooks et al., 2013).

Chloramphenicol

Chloramphenicol is a bacteriostatic and protein synthesis inhibitor. This is achieved through inhibition of the peptidyl transferase activity of bacterial ribosomes thus preventing peptide bond formation.

Ciprofloxacin

Ciprofloxacin is a synthetic chemotherapeutic antibiotic belonging to the second generation of fluoroquinolones drug class. It is a broad-spectrum drug that is active against both gram-positive and gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase and type IV topoisomerase enzymes necessary to separate bacterial DNA thereby inhibiting cell division (Hoerr et al., 2016).

Gentamicin

Gentamicin is an aminoglycoside synthesized by Micromonospora a Gram-positive bacterium that is widely present in water and soil. It is among the few heat-stable antibiotics and even stable after autoclaving which makes it useful for the preparation of particular microbiological media.

Gentamicin is a bactericidal antibiotic that works by binding the 30s subunit of the bacterial ribosome thereby interrupting protein synthesis(Hoerr et al., 2016).

Ampicillin

Ampicillin is a beta-lactam drug belonging to the penicillin group of antibiotics. It was among the first broad-spectrum penicillin introduced by Beecham. It demonstrates activity against Gram-negative bacteria such as coliforms, Haemophilus influenza and Proteus species. It differs structurally from other penicillin only by an amino group hence called aminopenicillin.

Ampicillin can penetrate the Gram-positive and Gram-negative bacteria. The amino group helps the drug penetrate the outer membrane of the Gram-negative bacteria. Ampicillin acts as a competitive inhibitor of transpeptidase which is an enzyme needed by bacteria in their cell wall synthesis. It inhibits the third and final stage of cell wall synthesis in binary fusion which leads to cell lysis(Hoerr et al., 2016).

Nalidixic Acid

Nalidixic acid is a synthetic 1,8-naphthyridine antimicrobial agent with a limited bacteriocidal spectrum. It is an inhibitor of the A subunit of bacterial DNA gyrase.

Antibiotic Resistance

It is the ability of bacteria to resist the effect of an antibiotic that it was previously sensitive to. Antibiotic resistance is a challenge due to increased antibiotic-resistant infections in both animals and humans (Prestinaci et al., 2015). The widespread use of antibiotics is one of the main reasons for the occurrence of antibiotic-resistant strains in the environment (Barton, 2000). In poultry production particularly in Wakiso district, there has been intense use of antibiotics where 96.7% of farmers were reported to use antibiotics on their farms and 33.3% of them used as growth promoters. Tetracycline (73.3%) were the most used followed by sulphonamides (26.7%) (Bashahun GM & Odoch, 2015). Other commonly used antibiotics are ciprofloxacin, gentamicin, chloramphenicol, and nitrofurans. Due to repeated exposure to the antibiotics, bacterial resistant strains have evolved and the use of antibiotics could be one of the reasons for the emergence of bacterial resistant strains.

Some bacteria are naturally resistant to bacteria. However, bacteria may become resistant through genetic mutation or by acquiring resistance from other bacteria. Mutations are spontaneous genetic changes that may lead to the evolution of antibiotic-resistant genes within a bacterial genome (Eng et al., 2015). These genes may be responsible for the production of enzymes that inactivate the antibiotic, eliminate the antibiotic target from the bacteria, close up entry ports for the bacteria or even manufacture pumping mechanisms that eliminate the antibiotic from the cell thus the antibiotic never reaches the target.

Bacteria may acquire antibiotic-resistant genes from other bacteria through transformation, conjugation and transduction. In transformation, naked DNA is passed to the recipient. Transduction takes place where a bacteriophage transmits the antibiotic-resistant traits into the bacteria. The traits are packed in the head of the virus and then it injects them into the recipient bacteria. In conjugation, the bacteria can transfer genetic material, including antibiotic-resistant genes (found on plasmids and transposons) from bacteria to another via a bridge formed during cell to cell contact. Bacteria like Salmonella and E. coli have developed different mechanisms by which they can resist to a commonly used antibiotic such as alteration of an antibiotic agent, a mutation in the target site, decreased uptake and increased efflux.

Causes of Antibiotic Resistance

There has been a rapid emergence of antibiotic-resistant pathogens worldwide reducing the efficacy of available antibiotics. The evolution of antibiotics is however attributed to several different factors.

Antibiotic overuse is one of the major reasons for the evolution of antibiotic-resistant pathogens(Read & Woods, 2014). However, this problem was earlier detected in 1945 by Sir Alexander Fleming warned that the ‘‘public will need the drugs and then begin an era of abuse'' (Bartlett, Gilbert, & Spellberg, 2013).

Inappropriate subscription is also linked to the emergence of antibiotic-resistant bacteria. Sub-therapeutic antibiotic concentrations can lead to antibiotic resistance through genetic alterations such as a change in gene expression of the bacteria and also mutagenesis (Viswanathan, 2014).

Extensive antibiotic use in agricultural production is a major factor for the rise of antibiotic resistance. An estimated 80% of the antibiotics used in the US are used in animal production as growth promoters and to prevent infections (Spellberg & Gilbert, 2014). In this way, antibiotics are ingested by humans usually in sub-therapeutic levels from farm products hence leading to evolution to antibiotic-resistant strains in humans via the food chain.

Public Health Concern for Broiler Production Practices

Poultry is considered to be a reservoir of E. coli and Salmonella capable of causing infections in humans. In an

unfortunate linkage, chicken products are suspected to be a source of foodborne ExPEC and Salmonella infections in humans. Furthermore, there has been the emergence of multidrug resistance (MDR) (resistance to three or more classes of antimicrobial agents) among avian E. coli that have created major economic and health concerns, affecting both human healthcare and poultry industries (Mellata, 2013).

The use of antibiotics in poultry production will select for drug-resistant bacteria. Among the various uses for antibiotics, low-dose, prolonged courses of antibiotics among food animals create ideal selective pressures for the propagation of resistant strains. The spread of resistance may occur by direct contact or indirectly, through food, water, and animal waste application to farm fields (Marshall & Levy, 2011). Through direct contact, farmworkers and veterinarians are at risk of contracting infectious agents directly from infected birds. The first evidence was reported by Levy et al., who isolated the same tetracycline-resistant E. coli strains from the gut flora of chicken caretakers as in the chicken feed on tetracycline supplemented feeds (Levy, Fitzgerald, & Macone, 1976). Another study among US poultry workers revealed the risk of carrying gentamicin resistant E. coli was 32 times more in poultry workers than in other people in the community (Price et al., 2007).

From the above possibilities, broiler production could be one of the sources of antibiotic-resistant pathogens causing infections in humans. This has led to increased mortalities in the country due to these infections.

METHODOLOGY

Research Design

It was a cross-sectional quantitative study aimed at determining the prevalence and antimicrobial susceptibility of Escherichia coli and Salmonella isolates from feeds, litter and cloacal swabs from broiler hens in Kalerwe and Kasubi markets of Kampala.

Study Area

The study was carried out in Kalerwe and Kasubi markets of Kampala. These markets are among the principal suppliers of broilers to the population of Kampala. Kalerwe market is located along Gayaza road adjacent the Northern By-pass about 5-kilometres from Kampala city centre. Kasubi market is located along Kampala Hoima road within 5 kilometres from Kampala city central business district. Kasubi market is one of Kampala's largest food markets situated opposite the road of Kasubi Tombs. The markets are supplied by many intensive broiler farms in and around Kampala hence samples collected from these markets was a representation of a large number of broiler farms.

Study Population

The study focused on broilers chicken contained in cages and ready for consumption. These broilers are obtained from various poultry farms in and around Kampala. They are feed in the cages as they await for sale in Kalerwe and Kasubi market. There is limited statistical information on the approximate number of broilers sold in Kalerwe and Kasubi markets.

Study Selection Criteria

Inclusion Criteria

Healthy broilers ready for human consumption were used to obtain cloacal swabs. Feed samples only from the stock were collected. Feeds mixed with fecal matter in the broiler cages were collected as litter samples.

Exclusion Criteria

Broiler chicken whose owners denied sampling were not sampled.

Sample Size Determination

$n= (Z\alpha + Z\beta)2 X P(1-P) / \delta 2$

The sample size will be calculated using Kish-Leslie formula (1965) below

Where n = the desired sample size.

$Z\alpha$ = the standard normal deviation 1.96, at 95% confidence interval.

$Z\beta$ = the standard normal deviation 0.84

P = estimated prevalence of E coli in litter (0.875)(Islam et al., 2014).

δ = maximum error the investigator is willing to allow, (5%).

Therefore, n = (1.96+0.875)2X 0.875(1-0.875)/ (0.05)2

= 343

Although the sample size calculated was 343, only 180 samples were collected due to financial constraints.

Samples were randomly collected broiler cages and an equal amount was obtained from each of the markets as shown in the table below.

SAMPLE	Number of samples		Total
	Kalerwe	Kasubi	
Feeds	30	30	60
Cloacal swabs	30	30	60
Litter	30	30	60
Total	90	90	180

Sample Collection

Feed and litter samples were randomly collected from commercial broiler cages of Kalerwe and Kasubi market. Cloacal swabs were collected from broiler chicken at the age of consumption in the cages from which the feed and litter samples were obtained.

Collection of Cloacal Swab Samples

Cotton tipped sterile swabs were dipped in normal saline and then used to aseptically swab the cloacal of the chicken. The swabs were then put on ice in the sample transportation box.

Collection of Feed and Litter Samples

Approximately 10gm for each litter and feed sample were collected in sterile sample containers. Feed samples were obtained from the stock feeds. Litter samples were obtained from the cages in which the broiler chicken were reared.

Transportation of Samples

Well labeled samples were placed on ice in a cooler bucket to maintain the sample integrity. The samples were then immediately transported to the Mulago Hospital Microbiology laboratory for processing and analysis.

Laboratory Analysis

Labeled samples were brought to the laboratory and then given unique laboratory numbers. The laboratory numbers were entered into the data collection book.

Preparation of the Inoculums

Litter and feed samples collected in sterile plastic containers were diluted in sterile normal saline and kept for 1hr in a sterile environment. Then 1ml of each of the sample was incubated in 9ml of nutrient broth for enrichment and incubated overnight at 37oC. Cloacal swabs were also independently and thorough mixed in 9ml of nutrient broth and then incubated overnight at 37oC for enrichment.

Determining the Prevalence of E. coli.

Laboratory Isolation of E. coli

A loopful from the broth of each sample inoculum was streaked on to freshly prepared chromocult agar. The inoculated plates were incubated at 370c for 24hrs in an aerobic incubator. Chromocult agar is a selective media for E. coli, which produces blue colonies on the media.

Identification of E. coli.

The identification of E. coli primarily depended on colony characteristics that are dark blue to violet-colored colonies. Colonies were subjected to microscopic examination after Gram staining to observe the morphological characteristics and their staining characteristics. E. coli cells appeared as typical rods, pink(Gram-negative) in color. Colonies with the above characteristics were confirmed to be E. coli and then transferred to fresh chromocult agar to obtain a pure culture for each positive sample.

SAMP LE	FORMULA FOR PREVALENCE
Feed	Number of positive feed samples/total number of feed samples x 100
Litter	Number of positive litter samples/total number of litter samples x 100
Cloacal swabs	Number of positive cloacal samples/ total number of cloacal samples x 100
Overall samples	Number all positive samples/total number of samples x 100

Table 1: Table showing how to calculate the prevalence.

Determining the Prevalence of Salmonella

Laboratory Isolation of Salmonella

A loopful of each sample from the broth media was collected and inoculated on to freshly prepared XLD agar and the incubated at 37oc for 24hrs. Colonies that appeared red with black dots in the center were inoculated onto fresh XLD agar.

Morphological Identification of Salmonella

Morphological identification of Salmonella primarily depended on colony characteristics on XLD agar where Salmonella colonies appeared red with black dots in the centre. A Gram smear was made using presumptive colonies and examined under a light microscope. Salmonella appeared as pink rods(Gram-negative).

Biochemical Identification of Salmonella

The biochemical identification of Salmonella colonies was based on TSI, Citrate and Urease tests. Presumptive colonies from the XLD pure culture were directly stabbed through the center of the TSI medium to the bottom of the tube and then streaked on the surface of the agar slant. The innoculate samples were incubated with a loosen cap for 24hrs at 370c. This was applied to test the ability of microorganisms to ferment sugars and produce hydrogen sulphide.

For the urease test, 2 loopful of pure and well-isolated colonies were inoculated into the urea agar by stabbing the butt and streaking the slant. The inoculated tubes were shaken gently and incubated with loosened caps for 24hrs at 35oC in an incubator. This was applied to asses the ability of the isolates to split urea through the production of urease enzyme that breaks down urea to produce ammonia causing alkalinity of the media thus changing the indicator to light orange.

For citrate test, a sterile loop was used to inoculate part of the colony in the test tube containing citrate agar by stabbing the butt and streaking on the slant and incubated at 370c for 24 hours. This was used to assess the ability of the isolates to use citrate as the sole source of carbon and energy. The utilization of citrate and ammonium salts in the citrate medium by the isolates leads to production of ammonia which increases the PH changing its colour to Prussian blue

The TSI agar was checked for the hydrogen gas production and alkalinity while the urease test was checked for the degradation of urea. Isolates that caused the darkening of the butt, red coloration of the slatt and produced hydrogen gas on TSI agar, utilised citrate and produced no change in the Urea agar were confirmed as Salmonella.

Determining the Antibiotic Susceptibility of Salmonella and E. coli

Antibiotic susceptibility of E. coli and Salmonella isolates to different agents was determined in vitro by employing a disc diffusion test of Kirby-Bauer method. Antibiotics used in the

study were ampicillin (10µg), ciprofloxacin (5µg), chloramphenicol (30µg), tetracycline (30µg), gentamicin (10µg) and Nalidixic acid (30µg). Cartridges of antibiotics containing discs were always stored at 4oc and allowed to come to room temperature before use. Isolates were transferred to 5ml sterile 0.9% saline to match with the 0.5 Mac Farland standard. A sterile cotton-tipped swab was used to streak air-dried Mueller Hinton plates within 15min of adjustment of turbidity. Subsequently, antibiotic discs were added and plates were incubated aerobically at 37oc for 18hrs. The diameter of zones of inhibition surrounding the antibiotic discs were measured in mm. Isolates were classified as resistant, intermediate and sensitive based on the breakpoints recommended by the guidelines of NCCLS.

Data Quality Control

- All laboratory procedures were performed according to the available Standard Operating procedures (SOPs).
- Standard E. coli (ATCC 25922) and Salmonella (ATCC 13076) were used to quality control gram staining and biochemical tests.
- Freshly prepared culture plates were randomly selected from each batch for overnight incubation at 370 C to check for sterility.
- Performance testing was done by inoculating standard Salmonella and E. coli on to XLD and Chromocult agar respectively.
- The media was autoclaved before being poured on to the plates for purposes of sterility of the media.
- Colony identification was done with the aid of a microbiological expert in the laboratory.

Data Analysis

Descriptive statistics were used, that is frequencies and percentages for different samples (litter, feeds & cloacal swabs) were obtained. A comparison was done for the two markets (Kalerwe and Kasubi) using the chi-square-test and significance will be considered at P≤0.05. SPSS was used.

Susceptibility patterns were presented in inform of percentages, frequencies and graphs. SPSS version 23 was used.

Ethical Consideration

Informed consent was sought from all the market vendors whose broiler chicken were to participate in the study. Confidentiality during study was maintained. The refusal of the market vendors to allow sampling of their broiler chicken, feeds and litter was respected and considered.

Results

The Overall prevalence of Salmonella and E. Coli in Samples Obtained Kasubi and Kalerwe Markets

Table 3 below shows the frequency and percentage of positive E. coli and Salmonella samples collected from the two study markets. Of the samples collected from both markets, E. coli and Salmonella isolates were recovered in only 126 of 180 (70%, 95%CI 62.8-76.3) and 3 of 180 (1.67%, 95%CI 0.5-5.1) samples respectively. The overall prevalence of E. coli and Salmonella in the Kasubi market was 84.4% and 2.2% respectively and that in Kalerwe market was 55.6% and 1.1% respectively.

Organism	N	Frequency	%	95% CI
E. coli +	180	126	70.0	62.8-76.3
Salmonella#	180	3	1.67	0.5-5.1

Table 3: Overall prevalence of E. coli and Salmonella isolates obtained from both Kalerwe and Kasubi markets.

+ Kalerwe = 55.6% (50/90), Kasubi = 84.4% (76/90)

Kalerwe = 1.1% (1/90), Kasubi = 2.2% (2/90)

Sample type	No of samples tested	No of positive samples	
		E. coli (%)	Salmonella (%)
Feed	60	31(51.7%)	0(0%)
Litter	60	42(70%)	1(1.7%)
Cloacal swabs	60	53(88.3)	2(3.3%)

Table 4: Distribution of E. coli and Salmonella among different sample types.

Prevalence of E. coli and Salmonella in the Litter, Feeds and Cloacal Swabs of Kalerwe and Kasubi Markets

As shown in Table 5 below, in each of the 30 feed, litter and cloacal swab samples collected from Kalerwe market, E. coli isolates were recovered from 8 feed, 14 litter and 28 cloacal swab samples. The prevalence of E. coli in Kalerwe feed, litter and cloacal swab samples was 26.7%, 46.7% and 93.3% respectively. Accordingly, cloacal swab samples from Kalerwe market had a significantly higher likelihood of E. coli isolation. (OR=38.5, P<0.001).

In each of the 30 feed, litter and cloacal swab samples collected from Kasubi market, E. coli isolates were recovered from 23 feed, 28 litter and 25 cloacal swab samples. The prevalence of E. coli in Kasubi feed, litter and cloacal swab samples was 76.7%, 93.3% and 83.3% respectively.

Salmonella isolates were recovered from only 1 of the 30 cloacal swabs and none from either feed and litter samples collected from Kalerwe market. Therefore the prevalence of Salmonella in Kalerwe cloacal swab samples was 3.3%.

Salmonella isolates were recovered from 1 litter and cloacal swab samples for each of the 30 respective samples collected from Kasubi market. However, none of the 30 feed samples obtained from Kasubi market was found to have Salmonella. The prevalence of Salmonella in the feed and cloacal swab samples obtained from Kasubi market was both 3.3%.

Organism	Place	Sample	N	Frequency	%	OR	95% CI	P-value
E. coli	Kalerwe	Feed	30	8	26.7	1		
		Litter	30	14	46.7	2.41	0.82-7.10	0.11
		Cloacal swabs	30	28	93.3	38.5	7.42-199.9	<0.001
	Kasubi	Feed	30	23	76.7	1		
		Litter	30	28	93.3	4.26	0.81-22.53	0.09
		Cloacal swabs	30	25	83.3	1.52	0.42-5.47	0.52
Salmonella	Kalerwe	Feed	30	0	0			
		Litter	30	0	0			
		Cloacal swabs	30	1	3.3			
	Kasubi	Feed	30	0	0			
		Litter	30	1	3.3			
		Cloacal swabs	30	1	3.3			

Table 5: Prevalence of E. coli and Salmonella in feed, litter and cloacal swab samples obtained from Kalerwe and Kasubi market.

Antibiotic Susceptibility Patterns of E. coli & Salmonella Isolates from both Kasubi and Kalerwe Markets

Table 6 below shows antibiotic susceptibility patterns of E. coli and Salmonella isolates to selected antibiotics. The isolates obtained were subjected to susceptibility testing using; Gentamicin (10µg), Ampicillin (10µg), Tetracycline (30µg), Chloramphenicol (30µg), Ciprofloxacin (5µg) and Nalidixic acid (30µg). The results of antibiotic susceptibility patterns of isolates from both Kalerwe and Kasubi markets indicate that; Most isolates E. coli isolates were resistant to tetracycline 73.8% (n=93) followed by ampicillin 70.6% (n=89). Most E. coli isolates were sensitive to gentamicin 83.4% (n=105) followed by ciprofloxacin 64.5% (n=81).

All Salmonella isolates were resistant to Nalidixic acid, chloramphenicol and ciprofloxacin.

DRUG	E. coli			Salmonella		
	Resistant	Intermediate	Sensitive	Resistant	Intermediate	Sensitive
Nalidixic Acid	71((56.3%)	17(13.5%)	38(30.2%)	3(100%)		
Gentamicin	13(10.3%)	8(6.3%)	105(83.4%)	2(66.7%)	1(33.3%)	
Ampicillin	89(70.6%)	20(15.9%)	17(13.5%)	2(66.7%)	1(33.3%)	
Tetracycline	93(73.8%)	27(21.4%)	6(4.8%)	1(33.3%)	1(33.3%)	1(33.3%)
Chloramphenicol	84(66.7%)	1(0.8%)	41(32.5%)	3(100%)		
Ciprofloxacin	21(16.7%)	24(19.0%)	81(64.5%)	3(100%)		

Table 6: antibiotic susceptibility pattern of E. coli and Salmonella isolates from both Kalerwe and Kasubi markets.

Antibiotic Susceptibility Patterns of E. coli and Salmonella Isolates from Kasubi and Kalerwe Market.

Figure one below shows: Most E. coli isolates from Kasubi market were resistant to tetracycline (n=71) followed by ampicillin (n=66) while those from Kalerwe market were most resistant to Chloramphenicol (n=50) followed by Nalidixic acid (n=38). Most E. coli Isolates from Kasubi market were sensitive to gentamicin (n=68) followed by ciprofloxacin (n=54) and then chloramphenicol (n=41) and those from Kalerwe, most were sensitive to gentamicin (n=37).

All Salmonella isolates (n=3) from both markets were resistant to nalidixic acid, chloramphenicol and ciprofloxacin. Only one isolate from Kasubi market was sensitive to tetracycline.

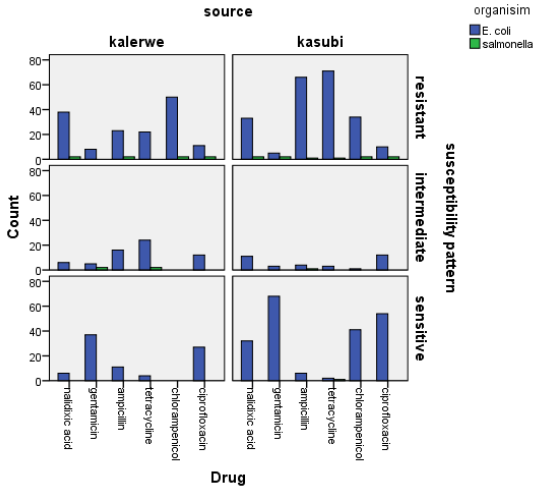

Figure 1: Antibiotic susceptibility pattern of E. coli and Salmonella isolates from Kalerwe and Kasubi.

Antibiotic Susceptibility Patterns of E. Coli and Salmonella Isolates from Litter, Feed and Cloacal Swabs

Figure 2 below shows: Most E. coli isolates from cloacal swab samples were resistant to chloramphenicol (n=38) followed by ampicillin (n=37) and tetracycline (n=36). However, most of these isolates were sensitive to gentamicin (n=48) followed by ciprofloxacin (n=33).

Most E. coli isolates from Litter samples were resistant to tetracycline (n=33) followed by chloramphenicol (n=31) and ampicillin (n=30). Most of these E. coli isolates were sensitive to gentamicin (n=31) followed by ciprofloxacin (n=27).

Most E. coli isolates from feed samples were resistant to tetracycline (n=24) followed by ampicillin (n=22). However, most of these isolates were sensitive to gentamicin (n=26) and ciprofloxacin (n=21). Generally, all E. coli isolates from either sample were more sensitive to gentamicin and ciprofloxacin.

All Salmonella isolates from cloacal swab showed resistance to nalidixic acid, chloramphenicol and ampicillin. The Salmonella isolates from the litter sample showed resistance to all the drugs. Most E. coli isolates from both feed and litter samples showed the highest sensitivity to gentamicin while those in cloacal swabs were more sensitive to ciprofloxacin.

All Salmonella isolates from cloacal swabs (n=2) were resistant to ciprofloxacin, chloramphenicol and nalidixic acid. Only one isolate was sensitive to tetracycline. The only Salmonella isolates from the litter samples were resistant to all antibiotics used in the study.

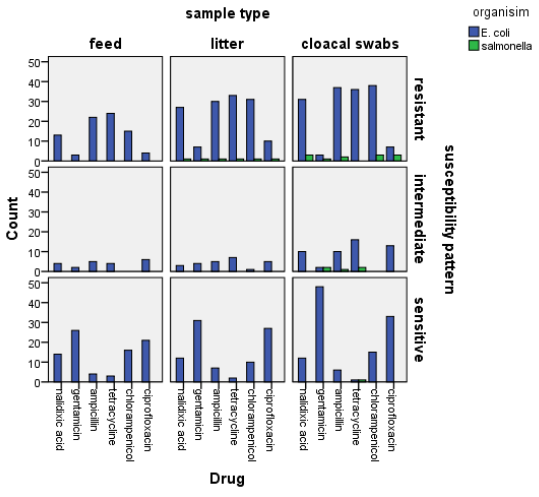

Figure 2: Antibiotic susceptibility patterns of E. coli and Salmonella isolates from litter, feed and cloacal swabs.

DISCUSSION

This cross-sectional study investigated the prevalence and antibiotic susceptibility of E. coli and Salmonella isolated from feeds, litter and cloacal swabs of broiler chicken sold in Kalerwe and Kasubi markets. The study found that the overall prevalence of E. coli and Salmonella was 70% and 1.67% respectively. This is in agreement with the prevalence of E. coli (70.83%) from broilers reported by Masuder et a.l, (2008). However, a slightly lower prevalence of E. coli (62.5%) and a higher prevalence of Salmonella (47.92%) was reported by Islam et al., (2014). The differences between the findings in this study and other studies could be due to the differences in environmental conditions and management practices. A slightly similar prevalence of Salmonella (1%) was reported by Hanson et al., (2002).

The market prevalence of E. coli was 55.6% for Kalerwe and 84.4% for Kasubi market. Then that for Salmonella was 1.1% for Kalerwe and 2.2% for Kasubi. There is a statistically significant difference between the prevalence of E. coli in Kalerwe and Kasubi market ($P<0.05$). This could be due to the differences in the source and handling of feeds (poor feed storage, use of dirty feed troughs) since feeds from Kasubi were more contaminated as compared to those from Kalerwe.

The prevalence of E. coli and Salmonella was highest in the cloacal swab (88.3% & 3.3% respectively) as compared to feeds and litter samples. This is because both E. coli and Salmonella are enteric organisms hence higher chances for isolation from the cloacal swabs. However, this significant high isolation rate justifies the possibility of contamination of feeds with fecal droppings. The prevalence of E. coli in cloacal swab samples (88.3%) in this study was higher than that reported by Akond et al., (2009). The overall prevalence of E. coli in feed and litter samples was 51.7% and 70% respectively. A higher prevalence of E. coli in the litter (87.5%) and a lower prevalence in feed samples from Bangladesh were reported by Islam et al., (2014). From a similar study, a higher prevalence of Salmonella in both litter and feed samples (29.16% and 66.66% respectively) was reported. The difference in the findings of the above study from the current one could be attributed to the differences in environmental conditions and hygiene on the poultry farms.

In the study, E. coli isolates were more resistant to tetracycline (73.8%) followed by ampicillin (70.6%), chloramphenicol (66.7%) and then to Nalidixic acid (56.3%). This is in agreement with the study carried out by Al-salauddin et al., (2015) that reported the highest resistance of E. coli isolates to tetracycline. Slightly higher resistance to tetracycline (94%), Nalidixic acid (100%) and almost a similar resistance to Chloramphenicol (67%) was recorded by Salehi & Bonab, (2006). Furthermore, higher resistance to tetracycline by E. coli isolates from Malaysian broiler chicken was reported by Apun et al., (2008). This could be attributed to the intensive use of tetracycline in the respective poultry farms as it is affordable as compared to other drugs.

E. coli isolates were more sensitive to Gentamicin (83.4%) and ciprofloxacin (64.5%). This is in line with the study carried out by Sarba et al, (2019) that reported a higher sensitivity of E. coli isolates to Gentamicin (93%). A relatively higher sensitivity of E. coli isolates to Gentamicin (97%) was reported by Salehi & Bonab, (2006). This is could be due to differences in the strains of E. coli. Similarly, this could be why there were differences in the antibiotic susceptibility patterns of E. coli isolates from Kasubi and Kalerwe markets.

Salmonella isolates were all resistant to ciprofloxacin, tetracycline and Nalidixic acid. The increased resistance of

Salmonella to ciprofloxacin and tetracycline is in line with the findings of Odoch et al., (2017). The increased antimicrobial resistance to these commonly used drugs in humans and animals poses a great public health challenge to Uganda. Similarly, in another study conducted by Mori et al., (2018) a highest resistance of Salmonella isolates to tetracycline (80.9% S. infantis & 83.9%, S. schwarzengrund) was reported. However, from a similar study, Salmonella isolates were all susceptible to ciprofloxacin which is contrary to what was reported from this study. The difference could be due to variations in the poultry disease management practices which involves unregulated use of antibiotics in poultry in Uganda which might not be the case in Japan.

The high level of antimicrobial resistance observed in this study is due to the widespread, indiscriminate, and lengthy use of similar drugs in the poultry farms as reported by Bashahun GM & Odoch, (2015). In support to this, information from some broiler chicken vendors, it is a common practice to treat sick chickens using drugs which they didn't know their name specifically that are obtained from the open market or veterinary/medical pharmacies. Such practice of using antimicrobials by untrained local people for treatment of chickens without proper diagnosis, selection of appropriate antimicrobial drugs, and strict adherence to proper dosage and frequency of administration accounts for the development of antimicrobial resistance. It might also be due to the widespread use of antimicrobials in humans and other livestock species, or incorrect use of antimicrobials by the rural people and chicken may ingest the antimicrobial residuals from human and animal wastes or due to improper disposal of leftover antimicrobials by rural people after getting relief from their disease.

CONCLUSIONS

The present study revealed a high prevalence of E. coli and a low prevalence of Salmonella in the samples collected with a higher isolation rate in cloacal swab samples. Furthermore, feeds and litter could be possible sources of bacterial contamination to broilers. A substantial proportion of E. coli and Salmonella isolates were found resistant to different classes of antimicrobial drugs which could be an important public health consequence.

RECOMMENDATIONS

Broiler feed troughs in cages should be firmly placed at a raised level to avoid possibilities of feed contamination with faecal droppings in broiler cages.

Proper disposal of waste should be emphasized to reduce the possibility of bacterial contamination of the environment surrounding these poultry markets since resistant E. coli and Salmonella were recovered from litter and feed samples that are usually recklessly disposed in the environment.

As a concern about resistant organisms isolated from poultry increases, it's important to educate public farmers on the risk and indiscriminate use of antibiotics in poultry production. Furthermore, the irrational use of antimicrobials and their availability on illegal markets should be addressed by establishing guidelines for the use of antimicrobials with effective enforcement.

More studies should be carried out in Uganda to identify the different strains of Salmonella and E. coli common in poultry and even identify genes of resistance common to those strains. This will enable effective management of poultry diseases.

STRENGTH AND WEAKNESS OF THE STUDY

STRENGTH: This study was the first to be conducted on broiler chicken sold in Kalerwe and Kasubi markets. In this way, broiler chicken raised from a number of poultry farms in Wakiso were sampled. This is because most of these farms sell their chicken to these markets from where the final consumers access them. Therefore samples obtained from these markets were a representation of the population in the poultry farms in Wakiso.

WEAKNESS: In this study, serotyping and identification of resistant genes in the isolates obtained was not done. However a number of studies have been done from which serotyping was done. For example S. infantis has been reported as the most prevalent serotype in broiler chicken of Kogoshima Japan (Duc et al., 2019). However, in the study carried out on Ugandan layer birds, reported Salmonella Newport as the most prevalent serotype (Odoch et al., 2017).

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