Veterinary Research 3 (4): 75-80, 2010
ISSN: 1993-5412

Antibiotic Susceptibility Profiles of Fecal *Escherichia coli* Isolates from Dip-Litter Broiler Chickens in Northern and Central Uganda

[1]Samuel Majalija, [1]Oweka Francis, [1]Wito G. Sarah, [1]Musisi-Lubowa,
[1]Patrick Vudriko and [2]Frances M. Nakamya
[1]Department of Veterinary Parasitology and Microbiology, [2]Department of Veterinary Anatomy,
Faculty of Veterinary Medicine, Makerere University, Kampala, P.O. Box 7062, Kampala

Abstract: The study aimed at determining the antimicrobial resistance profiles of *Escherichia coli* isolates from the fecal samples of broiler chicken reared under dip litter system in the northern district of Lira and central district of Kampala Uganda from February to March, 2010. *E. coli* was isolated on McConkey media and confirmed by standard biochemical tests. Antimicrobial susceptibility test was carried out by Disc diffusion test using 6 drugs; ampicillin, ciprofloxacin, chloramphenicol, cotrimoxazole, gentamicin and tetracycline. Out of the 220 collected faecal samples, *E. coli* isolates were recovered from 182 (83%) of these 90.8% (109) and 73% (73) were from chickens in Lira and Kampala districts, respectively. A majority of isolates, 168 of 182 (87%) showed resistance to at least one antimicrobial and most of these (103, 61%) were from Lira district. Of the isolates resistant to 2 or more drugs from both Lira and Kampala district, a majority were resistant to ampicillin, tetracycline in combination with cotrimoxazole. The emergence of multi-antimicrobial drug resistant *E. coli* in chicken from Uganda is probably due to inappropriate use of these drugs. There is need to implement measures which guard against misuse of antimicrobial drug in chicken production in order to minimize the emergence and dissemination of antibiotic resistant clones to the humans in close contact with the chicken.

Key words: Broiler production, prevalence, public heath, regulation, monitoring, chicken

INTRODUCTION

Chicken rearing is making an important contribution to the livelihoods of the most households in developing countries by providing food, income, employment and social (Van Eekeren *et al*., 2006). In Uganda, chicken production has steadily increased over the last 10 years from 22 million in 1999 to 37 million to date (MAAIF/UBOS, 2009). Chicken rearing enterprises are appealing to the low income earners particularly the women because of minimal inputs and the small unit area required to raise the birds. A majority of chicken in Uganda (90%) is local indigenous scavenging chicken reared under free range with minimal inputs (MAAIF/UBOS, 2009). However, with the increasing human population, rural to urban migration and improved livelihoods in the urban centres, the demand for animal proteins has increased exponentially. In response, a drastic shift towards intensive chicken rearing is taking place with increasing number of farms producing broilers and layer chickens in urban centres. The use of antimicrobials in sub-therapeutic doses has been suggested to improve the feed conversion efficiency of chicken, improve growth rate and allow them to develop into strong and healthy individuals with increase immunity against bacterial, viral and parasitic infections (NOAH, 2009). Farmers administer antimicrobial drugs in water or chicken feeds to treat or prevent infections (Food and Agriculture Organization, 2008). However, studies show that this practice promotes the emergence of resistant clones and resistance plasmids in strains of *E. coli* in the chicken which eventually infect people in close contact (Van den Bogaard *et al*., 2001). The transmission of resistant clones to humans renders treatment of infectious diseases less effective and increase mortality and morbidity in developing countries (Hart and Kariuki, 1998). Previous studies have reported inappropriate use of antimicrobials in animal production from Uganda (Byarugaba, 2004), a major factor in the emergence of antimicrobial resistance among microbes. Routine monitoring of antibiotic resistance provides data for antibiotic therapy and resistance control (O'Brien, 1997). Investigation of drug resistance in *E. coli* is of particular importance in understanding antimicrobial resistance of bacterial populations in general because this species occupies multiple niches including human and

Corresponding Author: Samuel Majalija, Department of Veterinary Parasitology and Microbiology, Faculty of Veterinary Medicine, Makerere University, Kampala, P.O. Box 7062, Kampala

animal hosts where genetic material exchange takes place (Levy, 1997). Nonetheless, little data is available on antimicrobial resistance patterns of bacterial pathogens in chicken from Uganda in general and to the knowledge the prevalence of antimicrobial resistant *E. coli* in broiler chicken from northern and central Uganda has not been documented. Therefore, this study investigated the antimicrobial susceptibility profiles of *E. coli* in broiler chicken under dip litter systems in the northern and central districts of Lira and Kampala, respectively. The data obtained will help in the designing interventions which will minimize the emergence and spread of antibiotic resistant bacteria in Uganda.

MATERIALS AND METHODS

Study area: The study was carried out in Kampala and Lira districts in central and northern Uganda, respectively from February-March 2010. Broiler farms were purposefully selected based on information from local poultry association leaders in each district. A guide assisted in the identification of farms. Only farms with >100 broiler chickens and were willing to participate in the study were included.

Study design and sample collection: This was a cross sectional study and the sample size was determined according to Kish (1996). Fresh fecal droppings were collected from 10% of the chicken from each farm. The pooled samples were placed in sterile plastic bottles each containing 30 mL of Stuart transport media. Immediately, the bottles were placed on ice in a cooler box and transported to the Microbiology Laboratory of the Faculty of Veterinary Medicine, Makerere University where they were placed at 4°C.

Bacterial culture and isolation: Bacterial cultures for the isolation of *E. coli* were prepared within 48 h of obtaining faecal samples. Faecal swabs were inoculated on MacConkey (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) and incubated at 37°C for 18-24 h. Indole, methyl red, Voges-Proskauer reaction and Simons citrate (IMViC) tests were performed with the colonies that showed growth characteristics of *E. coli*. Analytical Profile Index (API) 20E strips (Bio Merieux, Marcy-I'Etoile, France) were also used to confirm the identification of the isolates as *E. coli*. One isolate per sample was selected for resistance testing. The *E. coli* isolates selected for resistance testing were restreaked onto blood agar (Oxoid), incubated overnight at 37°C and stored at 4°C in 10% glycerol until *in vitro* susceptibility tests were performed.

Antimicrobial susceptibility test: Antimicrobial susceptibility testing was done by disc diffusion on Mueller-Hinton agar (Oxoid) as recommended by NCCLS (2001). The antibiotic discs used were of Tetracycline (30 µg), Ciprofloxacin (5 µg), Chlorampheni-col (30 µg), ampicillin (10 µg), cotrimoxazole (25 µg) and Gentamicin (10 µg) (Himedia Laboratories Ltd, Mumbai, India). The plates were incubated at 37°C for 18 h. The zones of inhibition were measured using callipers to the nearest millimeter and interpreted as susceptible, intermediate or resistant (NCCLS, 2001). *E. coli* ATCC 25922 was used as a reference strain for quality control of the antimicrobial susceptibility testing.

RESULTS AND DISCUSSION

A total of 220 faecal samples were collected from the 120 chicken farms in Lira district and 100 from Kampala district. Of these *E. coli* isolates were recovered in 109 (90.8%) and 73 (73%) from Lira and Kampala districts, respectively (Table 1). The overall prevalence of *E. coli* in broiler faecal samples was 83%. A total of 182 *E. coli* were tested for their sensitivity to six antimicrobials; tetracycline, Ciprofloxacin (CIP), Chloramphenicol (C), Ampicillin (AMP), Cotrimoxazole (SXT) and gentamicin (CN). Overall, 168 of 182 () isolates showed resistance to at least one antimicrobial. A majority of these 103 (61%) were from Lira and 65 (39%) from Kampala (Fig. 1). Significantly more *E. coli* from Lira were resistant to AMP, TET, SXT, C and CIP than those from Kampala.

None of the *E. coli* was resistant to CN (Fig. 1). A large proportion of *E. coli* from Kampala and Lira showed reduced susceptibility to Chlromaphanicol, 41 and 42%, respectively (Fig. 2). A significant number of *E. coli* isolates from Lira (42%) than Kampala (4%) showed intermediate susceptibility to gentamicin. On the other hand, more *E. coli* from Kampala (18%) were resistant to cotrimaxazole than those Lira (3%). Similarly, *E. coli* isolates from Kampala showed more intermediate susceptibility to tetracycline (24%) and ampicillin (10%) than those from Lira, although the different was not substantial. On the other hand, 6% of *E. coli* Lira showed reduced suscepitibility to ciprofloxacin as compared to 3% of those from Kampala (Fig. 1). In respect to resistance of *E. coli* to a single drug, slightly over one-third; 22 of 65 (34%) and 38 of 103 (37%) of the resistant *E. coli* from

Table 1: Isolation of *E. coli* from faecal samples of broiler chickens from Lira and Kampala districts

District	No. sampled	Isolation of *E. coli* (no. and percentage)
Kampala	100	73 (73)
Lira	120	109 (91)
Total	220	182 (83)

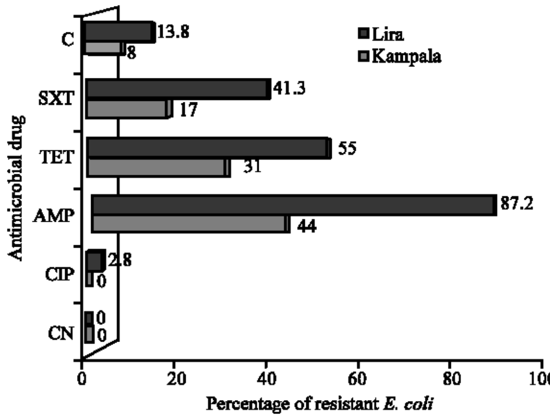

Fig. 1: Antimicrobial resistance profiles of feacal broiler *E. coli* from Lira and Kampala, CIP, Ciprofloxacin; C, Chloramphenicol; AMP, Ampicillin, SXT, Cotrimoxazole; CN, Gentamicin

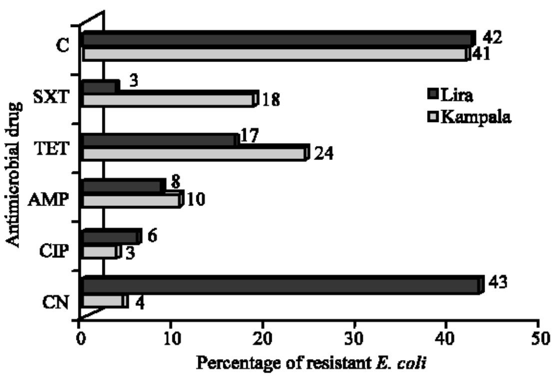

Fig. 2: Prevalance of broiler faecal *E. coli* showing reduce antimicrobial susceptibility from Lira and Kampala, CIP, Ciprofloxacin; C, Chloramphenicol; AMP, Ampicillin, SXT, Cotrimoxazole; CN, Gentamicin

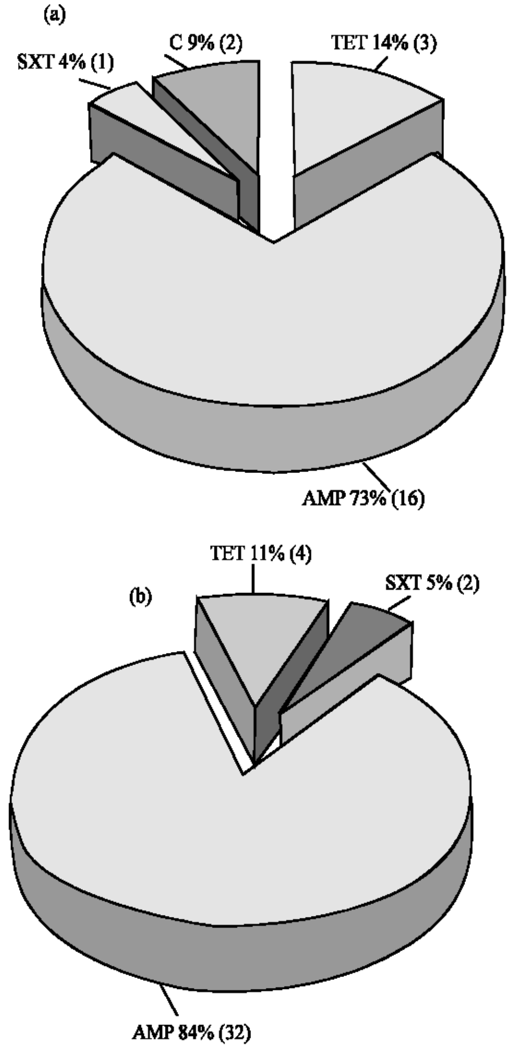

Fig. 3: Prevalence of mono-drug resistant *E.coli* from broiler chicken in Kampala (a) and Lira (b) districts C, Chloramphenicol; AMP, Ampicillin, SXT, Cotrimoxazole

broilers in Kampala and Lira district, respectively showed mono-drug resistance (Fig. 3a, b). A majority of these were resistant to Ampicillin; 73 and 84% from Kampala and Lira districts, respectively. About 14% of *E. coli* from Kampala and 11% from Lira were resistance to tetracycline.

Also 4 and 5% of the isolates from Kampala and Lira districts, respectively were resistant to cotrimoxazole. While only isolates from Kampala district (9%) showed resistance to chloramphenicol (Fig. 3). Regarding resistance to multiple antimicrobial drugs, a majority of resistant *E. coli*, 108 of 168 (65%) were resistant to two or more antimicrobials. These comprised 43 of 65 (66%) from broilers in Kampala and 65 of /103 (63%) from Lira district. Of these 58 and 38% *E. coli* isolates from Lira and Kampala districts, respectively were resistant to 2 antimicrobials (Fig. 4). On the other hand, *E. coli* isolates from chicken in Kampala were resistant to three (49%) and four (14) antimicrobials, more than from Lira district where the corresponding resistant isolates were 39 and 2% (Fig. 4).

Of the isolates resistant to 2 antimicrobials, a majority were resistant to ampicillin in combination with tetracycline in 25 and 42% from Kampala and Lira districts, respectively; Cotrimoxazole (8 and 7% in isolates from Kampala and Lira, respectively) or chloramphenicol in 5% of the isolates each from the two districts (Fig. 4). An additional 2.3% of the isolates which were each resistant tetracycline and Cotrimoxazole or chloramphenicol were isolated only from chicken in Lira district. Of the *E. coli* isolates resistant to three drugs, most were resistant to Ampicillin and tetracycline in combination with cotrimoxazole in 40 and 30% of isolates from Kampala and Lira districts, respectively (Fig. 5).

Other triple drug resistance profiles are resistance to ampicillin and chloramphenicol in combination with tetracycline or cotrimoxazole; tetracycline and ciprofloxacin in combination with cotrimoxazole or ampicillin and tetracycline, cotrimoxazole and chloramphenicol (Fig. 5). About 12 and 2% of isolates showed resistance to four drugs; ampicillin, tetracycline, cotrimoxazole and chloramphenicol from Kampala and Lira districts, respectively. The additional 2% of isolates from Kampala were resistant to a combination of ampicillin, tetracycline, cotrimoxazole and ciprofloxacin (Fig. 5). The dip-litter system where chickens are confined in houses with coffee husks and/or saw dust-covered floors is very popular in chicken rearing. Inevitably, confinement predisposes the chickens to diseases; bacterial and viral infections in particular (Compassion in World Farming, 2007).

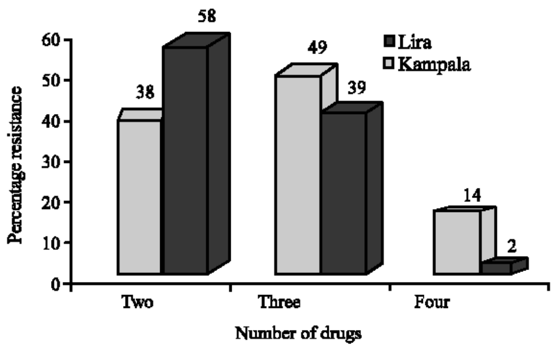

Fig. 4: Percentage of *E. coli* isolates resistant to two or more antimicrobial drugs broilers in Kampala and Lira districts

Antimicrobials used for therapeutic and prophylactic treatment of infectious agents contribute to the rise of antibiotic resistant bacteria in chicken and probably to farmers in close contact with chickens (Van den Bogaard *et al*., 2001; Hart and Kariuki, 1998). Nonetheless, information on antimicrobial resistant patterns of bacterial pathogens in broiler chicken under dipper litter system in Uganda is not available. Therefore, results from this study have shown the prevalence of antibiotic resistant *E. coli* in broiler chicken in the northern and central districts of Uganda.

Of the 220 chicken fecal samples, *E. coli* strains were not detected in 38 of 220 (17%) of faecal samples. This is not consistent with prior knowledge that *E. coli* is a normal gut flora in endotherms which is frequently shed in feces. One plausible explanation for this observation is the excessive use of antimicrobials for therapeutic and prophylactic treatment by the farmers. Over 90% of the

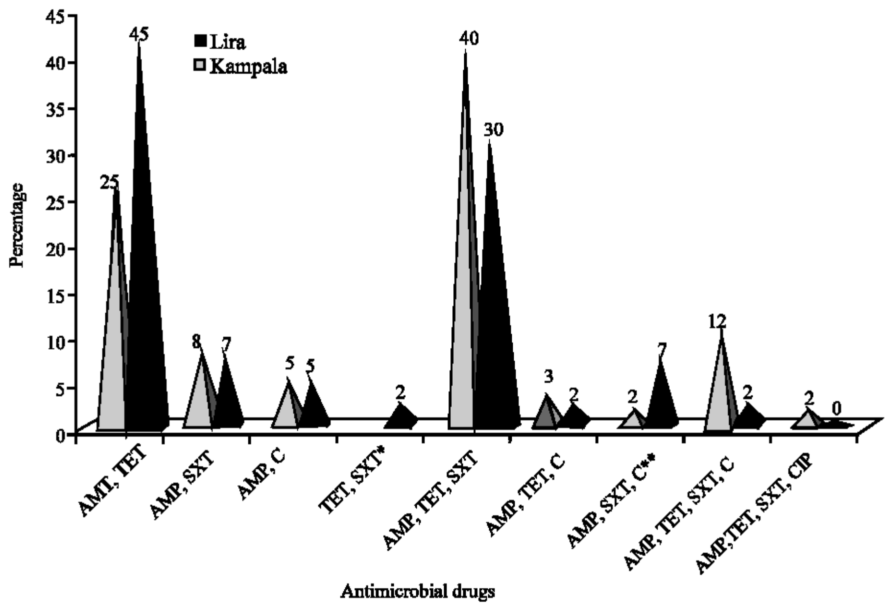

Fig. 5: Multiple drug profiles of broiler chicken *E. coli* isolates from Kampala and Lira districts, TET, Tetracycline; CIP, Ciprofloxacin; C, Chloramphenicol; AMP, Ampicillin; SXT, Cotrimoxazole; *2% of *E. coli* were each resistant to TET and C from Lira district only; **2% of *E. coli* from Kampala district were resistant to AMP, TET and CIP; TET, SXT and CIP or TET, SXT and C

antibiotics used in developing countries are for therapeutic purposes and not for growth promotion (Laxminarayan *et al.*, 2006). The prevalence of *E. coli* of 73% from the fecal samples in Kampala was <91% from chicken in Lira district. Although, birds in both districts were reared under the dip litter system, the antimicrobial use patterns were likely to be different. Other factors such as the different geographical location, environmental conditions and host factors are also known to affect the distribution of *E. coli* among animals of same species (Gordon and Cowling, 2003). Overall, the prevalence of *E. coli* in chicken in Uganda compares well with results from studies in Bangladesh (Akond *et al.*, 2009).

Available evidence shows that antimicrobial drug resistance is on the increase among commensal and pathogenic bacterial isolates from animals and humans (Salyers *et al.*, 2004; Linton *et al.*, 1988). Overall, 168 of 182 isolates showed resistance to at least one antimicrobial. There is an increasing tendency to overuse antimicrobial drugs by chicken farmers in Uganda probably due to aggressive marketing by the pharmaceutical industries. The farmers are lured to purchase antimicrobials with assurance of increased growth rates among their chickens. It was observed that more isolates from northern (61%) than the central region of Uganda (39%) were resistant to antimicrobials. The higher resistance can be attributed to sale of counter-feit drugs to unsuspecting farmers in the countryside where there is laxity in drug regulation. In developing nations over 60% of the drugs are probably counter-feits (Bryan, 2005) and this has been promoted the poorly regulated liberalized pharmaceutical sector. On the other hand, farmers in Kampala district have better incomes and can afford relatively expensive higher quality drugs for their flocks. Almost an equal percentage of *E. coli* isolates from northern and central Uganda which were resistant to one antimicrobial were resistant to ampicillin. This is expected because ampicillin is widely used for prophylactic and curative treatment of diseases of chicken in Uganda. A majority of *E. coli* isolates that were resistant to 2 or more antimicrobials cotrimoxazole from both districts were resistant to a combination of ampicillin, tetracycline and cotrimoxazole. Likewise, this was not surprising because the three drugs among the most widely used drugs to treat diseases of chicken either alone or in combination.

These results correspond to the findings of a similar study in Kenya (Mitema and Kikuvi, 2004) and Bangladesh (Akond *et al.*, 2009) where *E. coli* isolates showed multidrug resistance to ampicillin, cotrimoxazole and tetracycline. On the other hand, bacterial resistance to cotrimoxazole is of public health concern because this drug is being used to treat secondary infection in HIV/AIDS patients. In case of transmission of resistant clones of *E. coli* from poultry to humans, especially among the immune-compromised farmers there is a likelihood of complicated diseases which could result increase mortality and morbidity.

All isolates were susceptible to gentamicin, although this drug has been in use for a long time. This is probably because gentamicin has had limited use in chicken production (Bywater *et al.*, 2004) and the available parenteral preparations are not prescribed for use in chicken production. However, a significant proportion of strains showing reduced susceptibility to gentamicin suggest that there could off-label use of gentamicin by some farmers. Another explanation is that there might be a clone of *E. coli* with reduced susceptibility which has spread in the chicken population (Kijima-Tanaka *et al.*, 2003).

CONCLUSION

These results show that there is emergence of multi-antimicrobial drug resistant *E. coli* in chicken from Uganda probably due to misuse of drugs in chicken production. The antimicrobial resistance is of public health concern especially in disease-burdened communities. There is need for measures to minimize misuse of antimicrobials in chicken in order to contain the emergence and dissemination of antibiotic resistance and resistance genes to the humans in close contact with chicken.

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