ANTIBIOTIC RESISTANCE AND VIRULENCE FACTORS IN ESCHERICHIA COLI FROM BROILER CHICKENS SLAUGHTERED AT TIGONI PROCESSING PLANT IN LIMURU, KENYA.

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ABSTRACT

Background: Antibiotics and disinfectant use in broiler farms is a very common practice and an important risk factor for promoting the emergence, selection and spread of antimicrobial-resistant micro organisms in environment, veterinary and human medicine.

Objectives: To investigate multi-drug resistance and presence of virulence related genes in Escherichia coli isolates from healthy broiler chicken at slaughter time.

Design: Cross sectional and laboratory based study of virulence and drug resistance in E. coli

Setting: Tigoni processing plant, Limuru, Kenya.

Results: High resistance levels were detected for most commonly used drugs like tetracycline (75.9%) and cotrimoxazole (72.4%). Other antibiotics like ampicillin (39%), chloramphenicol (13.2%) and ciprofl oxacin (19%) recorded resistance levels although they are rarely used in poultry farming. One hundred and seventeen isolates showed resistance to two and more antibiotics. Different farm treatments were a significant factor for multidrug resistance (p< 0.001). The E. coli isolates showed twenty-one different multidrug resistant patterns with tetracycline/cotrimoxazole being the most common. Sixty samples were analyzed for virulence related genes using multiplex PCR. Seven virulence related genes were investigated but ten isolates were positive for verotoxin and three for intimin. Serotype 0111, 0126, 06 and 078 were positive for verotoxin,0126 and 0111 were positive for intimin. There was no significant relationship between virulence and multi-drug resistance (p< 0.05).

Conclusion: The present study highlights the presence of multi-drug resistant and virulent E. coli among healthy broiler chicken in Kenya. The possible source of antibiotic resistance in the broilers is the use of recommended antibiotics which co-select resistance for other antibiotics. Surveillance for drug resistance pathogens in food products is recommended.

INTRODUCTION

There is a growing concern at global level on increased prevalence of antibiotic resistance to both pathogenic and commensal microorganisms. It is now generally accepted that the main risk factor for this increase in resistance in pathogenic and commensal bacteria is the increased use of antibiotics in both animals and human. Approximately 90% of all antibiotics used for veterinary purposes are given to food animals like poultry, pigs and calves orally dissolved in drinking water. The antibiotic use in animals is not only for therapy and prevention of bacterial infection but also as growth promoters. Most studies have shown that after the introduction of antibiotics the levels of resistance of pathogenic and commensal bacteria increases. Their level of resistance is considered to be a good indicator of selective pressure to antibiotic use and resistance problems are therefore expected to increase in pathogenic bacteria (1, 2).

There is concern about the use of antimicrobials in poultry. The mode of administration of antibiotics in poultry which is oral predisposes the entero bacteria to all antibiotics administered even if the condition being treated is not gastrointestinal in nature. This kind of exposure to antibiotics may eliminate the
susceptible strains leaving the non susceptible to proliferate, which are then passed out in the faecal droppings to the environment.

In intensively reared food animals, antibiotics may be administered to whole flocks rather than individual animals, and antimicrobial agents may be continuously fed to food animals such as broiler and turkeys as antimicrobial growth promoters. Therefore the antibiotic resistance selective pressure for bacteria in poultry is high and consequently their faecal flora may contain a relatively high proportion of resistant bacteria (3).

*Escherichia coli* have been shown to be both pathogenic and commensal in nature and have the ability to colonise the gastrointestinal tract and other extra intestinal organs of humans and other animals (4). Different virulence factors are expressed by different strains of *E. coli*.

Virulence is determined by numerous factors such as adhesion, penetration into the cells, antiphagocytic activity and production of toxins. Virulent factors are identified either as extra-chromosomal or nuclear in origin (5).

Virulent genes are normally associated with large plasmids and transposons. Plasmids are mostly conjugative. These plasmids in addition often carry antibiotic resistance and heavy metal resistance genes and/or other pathogenic factors such as toxins (6). Also genes coding for enterotoxin and drug resistance have been identified on the same plasmid.

In previous research on resistant *E. coli* strains from chicken, it was observed that *E. coli* isolates demonstrated high multi drug resistance (6). Antibiotic resistance in *E. coli* has been reported to be high in most chicken isolates even in the local breeds which rarely receive antibiotic treatment (7, 8). Chicken has been observed to be a source of resistant *E. coli* strains that colonise humans and cause disease. Most of resistant *E. coli* strains in chicken are believed to acquire resistance due to antibiotic use in poultry farming.

A study done on the carriage of potentially pathogenic *E. coli* in chicken in Thika district, Kenya, demonstrated that healthy chickens may carry enteropathogenic *E. coli* strains (10). This study did not relate pathogenicity with antibiotic resistance, which the current study investigated. The work was also done on small-scale farms, while the current study sourced samples from large-scale broiler farms. The study also used multiplex PCR assay to detect the virulence genes. The previous study (10) used DNA-DNA hybridisation, cultured cell and transmission electron microscopy. Most of the work done has majored on the resistance levels with or without selective pressure. The virulent factors that confer extraintestinal conditions in humans in relation to resistance have also been studied well. Limited study has been done on prevalence of drug resistance and diarrhoeagenic *E. coli* strains from poultry. This study targeted gastrointestinal strains that could be a source of meat contamination during slaughter and on consumption gets their way into the human body.

**MATERIALS AND METHODS**

**Collection of caecal samples:** The samples were obtained from Tigoni processing plant in Limuru in the Central province of Kenya. This is the main supplier of broiler meat to most urban areas and the largest poultry processing plant in Kenya owned by Kenchic company. The company has contracted farmers to raise broilers for slaughter and distributes the meat to various outlets. The company supplies chicks and feeds to the farmers from the same source. The farmers were interviewed to get the information on the treatments given during the rearing period. All the farms reported use of disinfectants, anticoccidiostats and vitamins during the rearing period. Although some of the farmers reported antibiotic treatment of their birds some reported development of viral conditions but no treatment given (Table 1). The birds are kept for six weeks and ready for market. The caecal samples were collected at slaughter time from slaughtered birds. The specimens were transported at 4°C in a cool box from the slaughterhouse to the laboratory and processed within hours of collection.

**Isolation and identification of escherichia coli:** The caecum was aseptically opened using sterile scissors and a sterile wire loop used to pick the contents. The content was streaked onto MacConkey agar (Oxoid, Basingstoke, United Kingdom) for isolation of *E. coli*. The scissors and wire loop were flamed in between the inoculations to avoid cross contamination. Inoculated plates were incubated overnight at 37°C in ambient air. Pink circular, convex, smooth, non-viscous colonies with clear-cut margins suspected to be *E. coli* were purified and confirmed using analytic profile index (API) 20E strips (Biomerieux, Marcy-l’Etoile, France). The confirmed *E. coli* isolates were tested for antimicrobial susceptibility according to Clinical Laboratory Standard Institute recommended procedures *E. coli* A TCC 25922 (11) was used as reference strain and for quality control.

**Antimicrobial susceptibility testing:** The isolated *E. coli* was screened for anti-microbial susceptibility test using the disc diffusion method on Mueller-Hinton (MH) agar (Oxoid). The procedures were done according to the methods recommended by the Clinical and Laboratory Standard Institute Guidelines (CLSI; 2002). Antibiotic discs from Biomerieux-France and from Becton Dickinson-USA for tetracycline were
used. An antibiotic concentration on each disc was as follows, chloromphenicol 30mg, Ampicillin 10mg, tetracycline 30mg, Cotrimoxazole (23.75/1.25mg), ciprofloxacin 5mg and cefotaxime 30mg.

Detection of virulence-related genes: Virulence related genes were detected using multiplex PCR. Extraction of DNA was done as follows; overnight broth bacterial suspensions were adjusted to 0.5 McFarland standard in sterile deionised water, boiled for 10 min and centrifuged for 5 min at 10,000RPM high speed then 5mg of supernatant was used for PCR. Seven sets of primers were used in two multiplex reaction tubes. The first multiplex reaction used primers, MK-I and MK-5 for detection of verotoxin (vt) gene, ST-I and ST-2 for heat-stable toxin (st) gene, LT-I and LT-2 for heat-labile toxin (lt) gene and I-I, 1-5 for enteroinvasive (invE) gene.

The second multiplex PCR used sets of primers, aggRkas-l, and aggRkas-2 for enteroaggregative mechanism (aggR) gene, eae-4 for attaching and effacing (eaeA) gene and EASTOSDI, EASTOAS2 for astA gene. The PCR was performed in a PerkinElmer Gene Amp PCR system 9600-R (Roche Diagnostic GmbH, Mannheim, Germany) in a 50ml reaction mixture containing 5ml template DNA, 5ml x10 PCR buffer (20mM MgCl₂), 4ml of 2.5mM dNTP mixture (dNTP, dCTP, dTTP, dGTP), 0.3111 of primer and 0.3ml (5U/ml) of ampliTaq, adjusted to 50ml volume with deionized water. The sequences for the primers used in the PCR are as shown in Table 2.

Table 1
Primer sequence used and the target locus. (Pass et al., 2001)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers sequence</th>
<th>Product Size (nucleotides)</th>
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<tr>
<td>VT1</td>
<td>F: 5'-ACGTTAACACGGTGTTGCRGGGATC-3'</td>
<td>121</td>
</tr>
<tr>
<td></td>
<td>R: 5'- TTGCCACAGACTCGTGATGTCCTTATGAT-3'</td>
<td>102</td>
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<td>VT2</td>
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<td></td>
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<tr>
<td>eaeA</td>
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<tr>
<td></td>
<td>R: 5'- TCGA TCCCCA TCGTCACCAGAGG-3'</td>
<td>241</td>
</tr>
<tr>
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<td>360</td>
</tr>
<tr>
<td></td>
<td>R: 5'-CCA TTTCTTTGCTGCTGCA TC-3'</td>
<td>360</td>
</tr>
<tr>
<td>STI</td>
<td>F: 5'- TTTCCCCCTTTTT AGTCACTCAACTG-3'</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>R: 5'- GGGACAGA TT ACAAAAAAATGTTCAAC-3'</td>
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<tr>
<td></td>
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<tr>
<td>Eagg</td>
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<td>194</td>
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<tr>
<td></td>
<td>R: 5'- AGCTCTAAGGATGAAAATGTAAG-3'</td>
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Polymerase chain reaction was performed under the following conditions; denature at 94°C for 30s, annealing at 47°C for 60s and extension at 72°C for 90s for 25cycles. These remained as conditions for the first set of multiplex reaction. The second set of multiplex PCR was done under the same conditions except for annealing which was carried out at 50°C (12). The PCR products were separated by horizontal mini electrophoresis on 3% agarose gel at 100V and stained with 1mg/ml ethidium bromide. The PCR products were sized against a 100-bp ladder marker (Takara, Shuga, Japan).

Serotyping: The isolates that were found to be positive for virulence factors were serotyped to ascertain their sero groups. Serotyping all the samples was not cost effective since it has been found that 50% of E. coli isolates are non reactive with the O-antisera(12) The samples were serotyped using the standard method (4). Serotypes were determined with group O-antisera (Denka seiken Co.Ltd, Tokyo, Japan) according to the manufacturers instruction.

Data analysis: Chi-square was used to test for association between different farm treatments and prevalence of multi-drug resistance. Multi-drug resistance among the isolates was found to be associated with different farm treatments (p < 0.001).

The association between multi-drug resistance and virulence related genes was also tested by chi-square. The association between multi-drug resistances and virulence related genes was not significant at (p< 0.05).

RESULTS

A total, of 174 E. coli isolates were obtained from six farms that supplied their birds for slaughter during the study period (Table 2). This comprised of 58 from Kajiado, 79 from Thika and 27 from Kiambu and a mean of 29 isolates per site were obtained.
Figure 1 shows the antibiotic susceptibility profile of *E. coli* isolates. Out of the six farms, farm IV had the highest prevalence of resistance to almost all tested drugs. The farm IV isolates showed a resistance of above 20% to almost all the antibiotics except cefotaxime. Farm III and IV isolates had the highest resistance to tetracycline (96.4% and 93.5%) respectively. Farm I and II isolates had the highest resistance to cotrimoxazole (85% and 83.9%). All farms registered resistance higher than 30% to tetracycline and cotrimoxazole. Tetracycline resistance ranged from 44.4%-96.4% in all the farms. Figure 1. Resistance to cotrimoxazole ranged from 37%-85% in all the farms. Ampicillin resistance ranged from 14.8%-60.7%. The rest of the drugs tested registered low resistance in all the farms. Resistance to chloramphenicol ranged from 32%-35.5% and Ciprofloxacin resistance ranged from 16.7% - 58.1%. Cefotaxime was the most susceptible drug of all the drugs tested recording resistance of 3.2% and 5% respectively for farm I and farm II. The different farm procedures and management were found to be significant (*p*<0.001) (Table 3).

Figure 1. Antibiotic resistance of *E. coli* isolates from different farms

![Bar chart showing antibiotic resistance of E. coli isolates from different farms](image)

**Table 3**

<table>
<thead>
<tr>
<th>Procedures &amp; treatments</th>
<th>Antibiotics</th>
<th>Vitamins</th>
<th>Water</th>
<th>Feed company</th>
<th>Other animals</th>
<th>Source of chicks</th>
</tr>
</thead>
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<tr>
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<td>No</td>
<td>Yes</td>
<td>river</td>
<td>Unga</td>
<td>R,C,F</td>
<td>Ken chic</td>
</tr>
<tr>
<td>Farms II</td>
<td>No</td>
<td>Yes</td>
<td>piped</td>
<td>Unga</td>
<td>R,C,F</td>
<td>Ken chic</td>
</tr>
<tr>
<td>Farms III</td>
<td>No</td>
<td>Yes</td>
<td>borehole</td>
<td>Unga</td>
<td>R,C,F</td>
<td>Ken chic</td>
</tr>
<tr>
<td>Farms IV</td>
<td>Yes</td>
<td>Yes</td>
<td>borehole</td>
<td>Unga</td>
<td>R,C,F</td>
<td>Ken chic</td>
</tr>
<tr>
<td>Farms V</td>
<td>No</td>
<td>Yes</td>
<td>piped</td>
<td>Unga</td>
<td>R,C,F</td>
<td>Ken chic</td>
</tr>
<tr>
<td>Farms VI</td>
<td>No</td>
<td>Yes</td>
<td>piped</td>
<td>Unga</td>
<td>R,C,F</td>
<td>Ken chic</td>
</tr>
</tbody>
</table>

*R=ruminants, C=canine, F=fenile. The type of vitamin used was same for all farms provided by the ken chic company.*
Figure 2
The overall antibiotic resistance profile for E. coli isolates

AMP=Ampicillin, TET=Tetracycline, COT=Cotrimoxazole, CHL=Chloramphenicol, CIP=Ciprofloxacin, C1X=Cefotaxime

Figure 3
Multi-drug resistance E. coli isolates and the frequency of occurrence per farm

FI=Farm I, FII=FArm II, FIII=Farm III, FIV=Farm IV, FV=Farm V, FVI=Farm IV.
The overall percentage antibiotic resistance for *E. coli* isolates for the drugs tested is shown in Figure 2. High resistance rates for tetracycline, cotrimoxazole and ampicillin were observed. Out of the 174 *E. coli* isolates investigated, 68 (39%) were resistant to ampicillin, 139 (75.9%) to tetracycline, 126 (72.4%) to cotrimoxazole, 23 (13.2%) to chloramphenicol and 33 (19%) to ciprofloxacin. Cefotaxime resistance was very low only 0.6%.

High susceptibility to cefotaxime, ciprofloxacin and chloramphenicol was observed; with one hundred and seventy one (98.3%) isolates susceptible to cefotaxime, 139 (79.9%) to ciprofloxacin, 148 (85.1%) to chloramphenicol, 92 (52.9%) to ampicillin, 48 (27.6%) to cotrimoxazole and 14 (23.6%) to tetracycline. The isolates showed the lowest susceptibility to tetracycline.

Figure 3 shows that one hundred and seventeen isolates (67.2%) had multi-drug resistance to ≥2 antibiotics and 34 (19.5%) of the isolates showed resistance to only one of the antibiotics tested. Twenty three (13.3%) of the isolates showed no resistance to any of antibiotics tested. It was noted that most of the resistance involved two or more antibiotics indicating multi-drug resistance among the isolates. Multi-drug resistance among the isolates was also found to be associated with different farm treatments (P < 0.001) (Table 3).

*E. coli* isolates exhibited twenty-one different resistant patterns (Table 4) with tetracycline/cotrimoxazole pattern being the most frequent 41 (27.2%) followed by tetracycline/ampicillin/cotrimoxazole 32 (21.2%). Tetracycline/cotrimoxazole resistant pattern was observed in all the six farms. Tetracycline/Ampicillin/cotrimoxazole pattern was observed in five farms except for farm VI. Single resistance was common with tetracycline and the highest multi drug resistance pattern was with tetracycline/ampicillin/cotrimoxazole/chloramphenicol/ciprofloxacin.

Detection of virulence genes: Seven virulence related genes were investigated in sixty four isolates and ten isolates were positive for verotoxin and three for intimin. (Figure 4). Fifty four (83.8%) of isolates investigated were negative for the other genes tested. The verotoxin gene was observed in the ten (16.7%) isolates (six with both vtl and vt2 and four with only vt2). Only three (5%) isolates were positive for intimin (effacing and attaching) genes. The association between multi-drug resistances and virulence related genes was not significant at (p<0.05). The following serotypes were identified; 0 III two strains, 06 two strains, 0126 three trains, 078 one strain and two were non reactive.
DISCUSSION

In this study we isolated *E. coli* from 100% caecal content of healthy slaughtered broiler chicken. The antibiotic susceptibility data from this study showed that broiler birds in Kenya harbour *E. coli* resistant to various antibiotics commonly used in both veterinary and human medicine. This agrees with similar study done in Korea on commensal *E. coli* isolates from animals that were highly resistant to antimicrobial agents that are commonly used as feed additives or therapeutic agents (13). Multi-drug resistance and different farm treatments was found to be a significant risk factor (p≤0.05). The relationship between multidrug resistance and virulence factors was also assessed in the current study and found none.

The use of antibiotics and disinfectants in broiler farms is a very common practice. Antimicrobial usage is considered the most important risk factor promoting the emergence, selection and spread of antimicrobial-resistant micro organisms in environment, veterinary and human medicine (3). The potential for transfer of antimicrobial resistance from enteric bacteria of food animals to human population is a cause for concern (14). Contact with food animals or their excreta or consumption of foods of animal origin has been suggested as the main route for dissemination of antibiotic resistance from food producing animals into human populations (15).

High resistance levels were detected for most commonly used drugs like tetracycline (75.9%) and cotrimoxazole (72.4%) (Figure 3). Tetracycline is currently the most commonly used antibiotic in poultry. It is used directly or in combination with others for treatment, prophylaxis or as growth promoter. Potentiated sulphonamides are also in great use for treatment and prophylaxis of both bacterial infections and coccidiosis. Other antibiotics such as ampicillin (39%), chloramphenicol (13.2%) and ciprofloxacin (19%) recorded resistance levels yet not used commonly in poultry farming in Kenya (Figure 3).

The resistance to tetracycline and cotrimoxazole was highest in all the six farms. Farm VI registered high susceptibility for most drugs but still registered high level of resistance to the two drugs. All farms registered resistance higher than 30% to tetracycline and cotrimoxazole. Through out the rearing period the birds are given antibiotics for prophylaxis and some times for treatment of diseases. The enterobacteria and intestinal *E. coli* of the broilers are subjected to selective pressure continuously. The susceptible bacteria are killed and the resistant ones are selected depending on the resistant gene present. The resistance to tetracycline is plasmid mediated and the plasmid encodes for other properties (16).

Resistance to ampicillin, ciprofloxacin and chloramphenicol was observed with no apparent selective pressure. The resistance levels also varied greatly with different farms. There are rare poultry preparations in Kenya that have ampicillin or its analogs. Chloramphenicol is rarely used in poultry in Kenya and norfloxacin use was discontinued three years ago which is a quinolone like ciprofloxacin. Therefore no selective pressure was present for these drugs. The results indicate that there could be another source of resistance selective pressure apart from individual antibiotic use.

Most chloramphenicol resistant isolates were also resistant to other antibiotics (Table 4). The most common resistant combination was with tetracycline and ampicillin alone or with any others. Therefore the resistance to chloramphenicol observed could be as a result of co-selection. In other studies the factors governing the persistence of chloramphenicol resistance in the absence of specific selection pressure has been done (8, 15). Class I integrons has been found to be significantly more prevalent in strains with chloramphenicol resistant genes (cmlA), compared to strains without chloramphenicol resistant genes. It has been shown that most gene cassettes within the integrons were involved in resistance to trimethoprim. There is evidence that resistance that develops because of the use of trimethoprim in cattle and pigs, also contributes to the selection of chloramphenicol resistant strains of *E. coli*. Thus, it is possible that bacterial resistance to chloramphenicol in animals would persist despite a ban on the use of chloramphenicol in cattle and pigs (17). These results suggest that in the absence of specific chloramphenicol selection pressure, the cmlA gene is maintained by virtue of gene linkage to genes encoding resistance to antimicrobials that are currently approved for use in food animals (18). The use of sulphonamides potentiated with trimethoprim could have induced the selection of trimethoprim resistance gene which has a linkage to chloramphenicol resistance genes.

Isolates with chloramphenicol acetyltransferase gene (catI) have been found to be resistant to ampicillin and tetracycline (17) this may be the phenomenon in the current study to explain high-level multi drug resistance. This study showed that the isolates that were resistant to chloramphenicol were also resistant to ampicillin and tetracycline (Table 4). Since chloramphenicol acetyltransferase gene (catI) is plasmid mediated and the tetracycline resistant gene is also plasmid mediated then the two genes can be mediated by one plasmid. The tetracycline genes often occur on mobile genetic elements, such as plasmids or transposons. The continuous use of tetracycline in poultry could result into the persistence of chloramphenicol resistance.
in *E. coli* without its use. Co-selection occurs for genes that are found on the same plasmids or same resistant gene cassettes.

In this study ampicillin resistance was 39% and almost 90% of the ampicillin resistant isolates were also resistant to tetracycline. Ampicillin and its analogs are rarely used in poultry in Kenya. There was no specific selection pressure resulting directly from ampicillin as a drug. Studies done on intestinal *E. coli* have shown that antibiotic resistance plasmids can persist for up to seven weeks in absence of selective pressure. A particular plasmid that confers resistance to trimethoprim and ampicillin has been found to persist in absence of selective pressure pIP’1531. *E. coli* has inherent factors that inhibit β-lactam drugs but the resistance to ampicillin was not a single drug resistance rather it involved more than one drug. This may indicate that the resistance could be plasmid mediated. The birds were fed on vitamin formulations throughout the six weeks and the vitamin brands used were prepared with a prophylactic tetracycline dose. They were continuously exposed to tetracycline. This induced selection pressure to resistant bacteria that possess resistant genes. These results agree with data of a similar study done in Pennsylvania showing 75% of ampicillin resistant *E. coli* isolates were also resistant to streptomycin and tetracycline (19). The ampicillin gene (amp gene) of *E. coli* is carried on the same plasmid with tetracycline resistance gene (pBR322) (20). Genetic element like plasmids can carry several genes and any selection pressure to select for any of the genes on the plasmid selects for the other genes simultaneously.

In this study 67.2% of isolates were resistant to two or more antibiotics. This data agrees with similar data (21) reporting that *E. coli* from poultry that were feed on feeds-laced with tetracycline, demonstrated resistance to tetracycline, ampicillin and sulphonamides. Resistance was found encoded on transferable plasmids that emerged following use of just tetracycline (21). Other studies have also shown that continuous use of a single drug could result into multidrug resistant microorganisms.

The tetracycline genes often occur on mobile genetic elements, such as plasmids or transposons (21). Plasmids harbouring tetracycline resistance may also carry other antibiotic resistance and virulence related genes (22). A selective advantage for any gene carried on the plasmid may enhance the persistence of plasmid-bearing strains. This phenomenon, termed co-selection, is thought to have contributed significantly to the increase in multiple drug resistance in bacteria over the last 40 years (23).

The following serotypes were identified; 0111 two strains, 06 two strains, 0126 three strains, 078 one strain and two were non reactive. Serotypes 0111, 06 and 0126 have been implicated in verotoxin clinical diarrhoeagenic human cases (12). Serotypes 0126 have been isolated from chicken and found capable of producing disease in human. A study done in Saudi Arabia found serotype 06 from broiler chicken similar to that in the patients in the same province. These results suggested the possibility of chicken being a source of pathogenic *E. coli* to human. Serotype 0111 has been associated without breaks of enterohaemorrhagic diarrhoea. It has been classified as one of the classical enterohaemorrhagic *E. coli*. However 0111 and 0126 serotypes have also been isolated from patients with diarrhoea and were negative for verotoxins and effacing and attaching gene. Serotype 0111 has also been found to possess the enteropathogenic genes. 0126 serotype has been found to be positive for enterotoaggregative in other studies. Serotype 078 has been known to be an avian pathogenic *E. coli* and to cause extraintestinal conditions in human. Studies have shown that the production of verotoxins is not usually present in serotype 078. However this study shows the serotype positive for verotoxin.

Verotoxin producing *E. coli* acquire the bacteriophage that encodes shiga-toxins. The genes are carried by λ-like bacteriophages that are integrated into the chromosome of their *E. coli* host. Shiga-toxin producing *E. coli* evolves through a series of genetic events that includes acquisition of the bacteriophages encoding verotoxin gene and modification of the gene encoding for 0 antigen. It has also been reported that some of the enterohaemorrhagic *E. coli* may lose the verotoxin producing ability during the cause of colonisation and infection. This explains why some studies have demonstrated serotype 0111, 06 and 0126 inherently known to be negative for...
verotoxin production to be positive. Also this study reported serotype 078 as positive for verotoxin. That is not usually present. This study then agrees with other studies which have shown the emergence of pathogenic clones of E. coli. Bacterial evolution is an ongoing process that undoubtedly will lead to the emergence of other successful pathogenic clones of E. coli in future (24).

This study investigated the potential of healthy broiler chicken harbouring human diarrhoeagenic E. coli. The following virulent factors were investigated: enteropathogenic, enteroinvasive, enterohaemorrhagic, effacing and attaching, and enteropathogenic. Ten (16.7%) isolates tested positive for verotoxin and three (5%) isolates were positive for intimin (effacing and attaching) genes (Figure 4). There was no significant relation between the presence of virulent genes and the multiple drug resistance (P<0.05). Verotoxins and intimins are transferred by a bacteriophage and not plasmid. This may explain why there is no association between multidrug resistance and verotoxin genes.

All isolates positive for intimin genes were also positive for verotoxin genes. The other enteric/diarrhoeal virulence genes were not observed from the isolates. Seventy percent of the positive isolates were from the same farm and 30% from another. Only two farms reported positive results for virulent factors. This may indicate management and environment of the farms played a major role on the composition of the micro flora of the broilers.

Three isolates (5%) were positive for intimin genes. Intimin genes are produced by attaching and effacing E. coli strains. All the intimin positive isolates were also positive for verotoxin. There is an association between intimin production and the pathogenicity of verotoxin producing E. coli. In addition the toxin produced by the E. coli is a protein called intimin, which is responsible for intimate attachment of verotoxin producing E. coli to the intestinal epithelial cells. This strain causes histopathological alterations termed as attaching and effacing (A/E) lesions in the intestinal mucosa (25).

Serotype 0113 and 0126 were positive for intimin genes. Verotoxin producing E. coli evolves from other strains by acquiring bacteriophage that encodes for verotoxin. Some studies have concluded that verotoxin producing E. coli that has eae-genes evolved from enteropathogenic E. coli. There is also evidence that some strains acquire the pathogenicity island from other bacteria through horizontal transfer via plasmids, transposons or bacteriophage (26).

CONCLUSIONS

The present study highlights the presence of multidrug resistant E. coli among healthy broiler chicken in Kenya. The source of antibiotic resistance in the broilers is mainly due to selective pressure the intestinal flora is subjected to. The antibiotics used selects for resistant strains which possess genetic elements that have resistant factor for other antibiotics that are not in use. This was observed where the poultry isolates were resistant to human only antibiotics like chloramphenicol and ciprofl oxacin. These isolates could also transfer the properties to other bacteria in the gastrointestinal tract of the broilers or humans. This study also investigated the potential of healthy broiler chicken harbouring human diarrhoeagenic E. coli. Ten (16.7%) isolates tested positive for verotoxin and three (5%) isolates were positive for intimin (effacing and attaching) genes. The observation of the verotoxin and the intimin proteins from the isolates demonstrated that healthy broilers may be potential carriers of human diarrhoeagenic E. coli.

Having meat products free from contamination is not possible in practise. However the occurrence can be minimised by practising high standards of hygiene in steps of food production chain. Also all meat products from broilers should be hygienically prepared and properly cooked before consumption. Continued antimicrobial surveillance in poultry may be essential.

REFERENCES


