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# OCCURRENCE OF VIRULENCE GENES AMONG ESBL-PRODUCING *E. COLI* STRAINS IN LOCAL SCAVENGING CHICKEN

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## ABSTRACT

To the best of our knowledge, this is the first attempt to describe the occurrence of virulence genes distribution amongst ESBL isolates in scavenging local chicken in Tanzania. The study sampled 200 chicken from six vicinities in the Morogoro municipality. Forty two percent of APEC isolates from *E.coli* were found to be ESBL producers, which harbored the ff ESBL genes; TEM, CTX-M1, CTX-M 914, CTX-M825, CMY, MA and OXA. These were co-harbored with a total of 31 virulence genes. The most prevalent virulence factor found amongst ESBL isolates include *iss*, *tra T, Asta A* and *ibe A*. The *tra T* virulence gene recorded a positive correlation with ESBL genes CTX-M1, CTX-M825 and CTX-M945 and CMY whiles the *IbeA* virulence gene showed positive correlation to only MA and CTXM-1. Also there is a significant difference in the number of virulence genes found in the oral-pharynges of the chicken and those found in their cloacae, an indication that these seemingly healthy birds harbor virulence genes and could promote zoonosis. Further research needs to go into the the relationship between virulence factors and resistance genes since it still remains is complex issue

Key words:, cloacal, ESBL, oral-pharyngeal, virulence genes

## INTRODUCTION

*Escherichia coli* (*E. coli*) has a wide diversity of virulence factors is considered a heterogeneous group of pathogens with (11). It causes different clinical manifestations, such as air sacculitis, perihepatitis, pericarditis, sepsis, salpingitis, coligranuloma, and cellulitis (3). Avian cellulitis has a multifactorial disease resulting from management failures, immunosuppressive diseases, and skin lesions. It has caused significant economic losses to the poultry industry (41). Avian pathogenic Escherichia coli (APEC), belongs to the pathotype extraintestinal pathogenic Escherichia coli (ExPEC) (22, 29). Considering its profile of virulence genes and its capacity of acting as a reservoir of antimicrobial resistance genes, it has zoonotic potential and can be transmitted to humans through the food chain (1, 11).

Antimicrobial resistance is a serious health threat both in human and veterinary. The extensive use of antimicrobials in veterinary is the main cause of the occurrence of resistant microorganisms (20,31). One of the main resistance mechanisms of bacteria to antibiotics is the production of extendedspectrum beta-lactamase (ESBL). The mechanism of action of these enzymes is the hydrolysis of the b-lactam ring of antibiotics. By doing this they inactivate several antibiotics, including cephalosporin of third and fourth generations, penicillin, cephalosporin (ceftazidime, cefotaxime, ceftriaxone) and monobactam (aztreonam), (6, 44).

The detection of virulence factors and antimicrobial resistance is key to unravelling the epidemiology and allows designing measures for the prevention and control of the disease. The aimed of this study is to establish the co-existence and relations between virulence genes and ESBL resistance genes as well as their phenotypic resistance to antimicrobials, and ESBL-producing capacity of APEC isolates obtained from avian cellulitis lesions

## METHODOLOGY

**Study area and sample collection and Identification of *E.coli* isolates**

The Morogoro municipality. It has a total area of about 531.6 km2 and a population growth rate of 4.7% per annum. The percentage of the populace that is engaged in livestock keeping and

subsistence farmingis 33% (35). A total of 400 swabs were collected from six different locations within the Morogoro municipality. Procedures used were as described in the Bacterial Analytical Manual BAM 2007 (7). The organisms were grown on MacConkey and Blood Agar media to detect positive *Escherichia coli*. Samples were suspected to be *E. coli* based on morphological appearance. The suspected samples were confirmed by biochemical tests.

**Virulence factor profiling to detect APEC strains**

The positive *E. coli* were investigated for various virulence genes by multiplex PCR, with protocol based on (16). Isolates that contain four or more virulence genes were considered APEC isolates (30). The virulence genes that were screened include were in 5 categories; Iron Acquisition (*Chu A, Iro N, Irp 2, Iuc D, Sit chr,sit ep*.), Serum resistance (*Cvi/Cva, Iss, Omp A, Tra t*) Adhesins (*Pap C, Tsh*), Toxins (*Ast A, vat*) and Invasins (*Gim B,Ibe A*). The procedures were performed in 25µlreaction mixture. This includes: 12.5 µl ofTaq polymerase (Dream Tag PCR Master mix, Inqaba Biotec East Africa Ltd), 0.5 µl of each 100Mm dNTP, 0.1µl(100pmol) oligonucleotide primer pair, 6.9 µl of nuclease-free water and 4µl of template DNA. Primer concentration is 0.4 M. Conditions of the reaction mixtures include: 5mins at 95ºC initial denaturation,94ºC of denaturation for 30s, annealing at 56ºC for 30s, elongation at72ºC for 3minutes at 25 cycles, a final elongation at 72ºC for 10 minutes and a hold at 4ºC.

**Antimicrobial Susceptibility Testing and Phenotypic Detection of ESBL**

The Kirby-Bauer antimicrobial sensitivity test method was used to determine the isolates that were susceptible to cephalosporins and betalactams, potential ESBL producers. However, susceptibility test were carried on other drugs as well, thus a total of ten antimicrobial drugs were used. These include; augmentinin (30µg), imipenem (10µg), cephalothin (30µg), cefotaxime (30µg), ceftazadime (30µg), ceftriaxone (30µg). The zones of inhibition were measured and the resistance was recorded based on Clinical and Laboratory Standards Institute (12). Double disc synergy test: to confirm of ESBL producers As described by (42), the double disk synergy was performed on the suspected ESBL producers for confirmation. The disks of ceftazadime (30µg), ceftriaxone (30µg) and cefotaxime

(30µg) were place around an augmentin disk (30µg), 20mm apart, on a Mueller-Hinton Agar plated swabbed with the test isolate. Enhancement of the inhibition zone of the cephalosporin toward the augmentin disc was interpreted as positive for ESBL production (42).

Isolates that were positive for ESBL were screened to know the types of beta-lactamase (bla) genes they harbored. A total of eight samples were screened for the presence of 12 bla genes. The protocol was by (25). The reactions were carried out in a 25 µl reaction volume. This consist of 12.5 µl of taq polymerase, 1 µl each of the primer sequence, 5.5 µl of the Nuclease free water and 5 µl of the DNA template. The primer concentration was 0.4M.

**Analysis**

Statistical analysis was done by use of Microsoft excel 2003/7 and Epi. Info. Proportions of various characteristics were tested by use of the chi-square test (x2 ). The threshold for statistical significance was indicated in the table with a $P < 0.05$ reflected statistical significance. In biological analysis; the following software were employed: MEGA 7 (48), Sequencing products were analyzed on the National Centre for Biotechnology Information (NCBI) using the basic alignment search tool BLAST(36)



## RESULTS

**Phenotypic resistance to antimicrobials and ESBL production**

Significant resistance of the bacterial isolates was detected against nalidixic acid (87.5%) whiles sparing resistances were detected against CTX, STX, AUG and KF, all at 37.5%. Very low resistances were detected amongst CRO (25%) and CAZ (12.5%) whiles CN, CIP and IMI recorded no resistances at all (Table 1). Forty percent of the isolates were found to be multi-drug resistant.

**Table 1 – Profile of phenotypic resistance to antimicrobials, ESBL production and multidrug resistance (MDR) in Escherichia coli isolates from lesions of avian cellulitis.**

| Drugs | No (%) of isolates showing resistance n=8 |
|---|---|
| CRO (30µg) | 2 (25%) |
| CTX (30 µg) | 3 (37.5%) |
| CAZ (30 µg) | 1 (12.5%) |
| CN (10 µg) | 0 (0%) |
| STX (25 µg) | 3 (37.5%) |
| AUG (30 µg) | 3 (37.5%) |
| NA (30 µg) | 7 (87.5%) |
| CIP (5µg) | 0 (0%) |
| KF (30 µg) | 3 (37.5%) |
| IMI (10 µg) | 0 (0%) |

**Prevalence of ESBL genes**

A total of seven ESBL genes were detected in the study; TEM, MA, CTXM-1, CTXM-825, CTXM-914, CMY and OXA. The TEM (fig 3) was the most prevalent; it occurred in all the samples (100%). The least prevalent was CTXM-1 which was amongst 12.5% of the samples. CTXM-914, CMY2 and OXA genes(fig 2) were detected amongst 37.5%, 62.5% and 75% of the total samples respectively. Both MA and CTX-M 825 were each detected amongst 50% of the samples (fig 1).



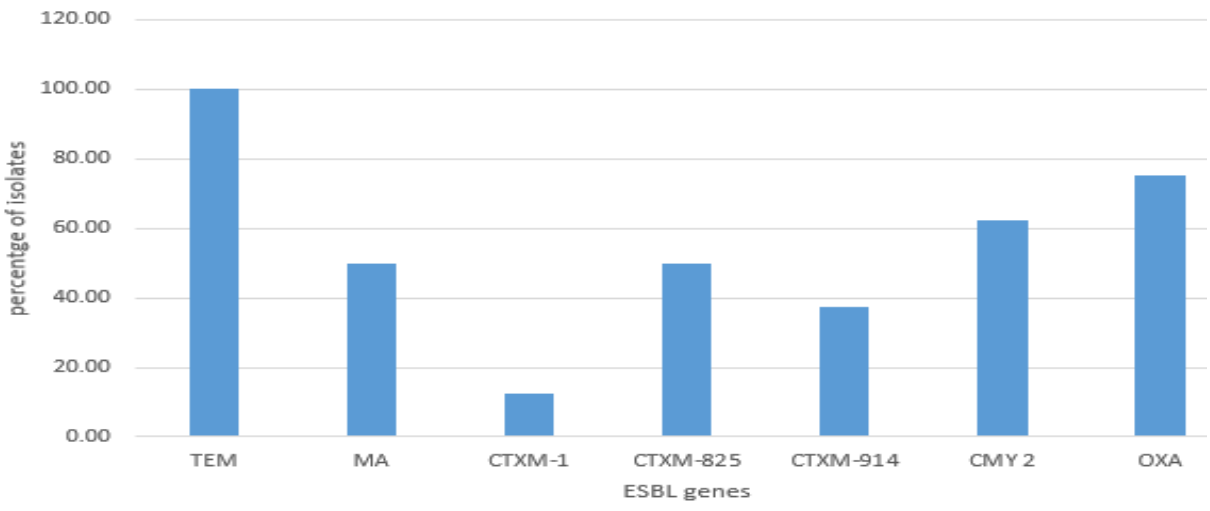


Fig 1: prevalence of ESBL genes amongst isolates

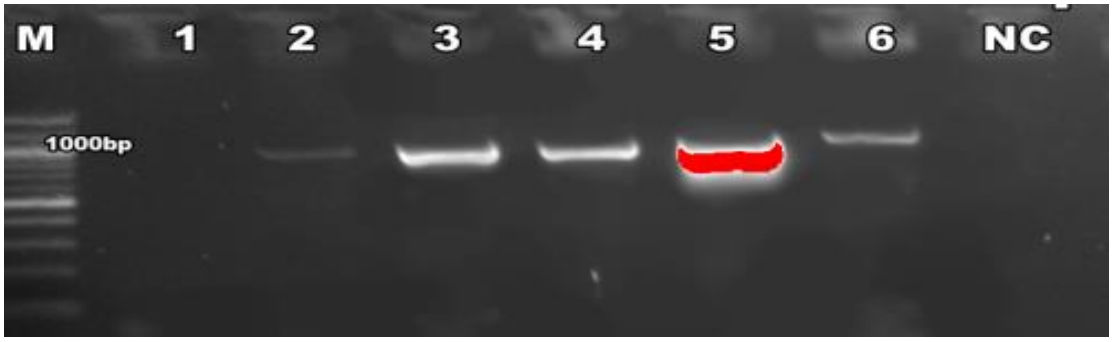


Fig 2:PCR detection of OXA gene (820 bp) PCR visualized under gel documentation. Lanes 2,3,4,5 and 6 are positive whiles 1 is negative. NC is negative control. M is marker (100bp).

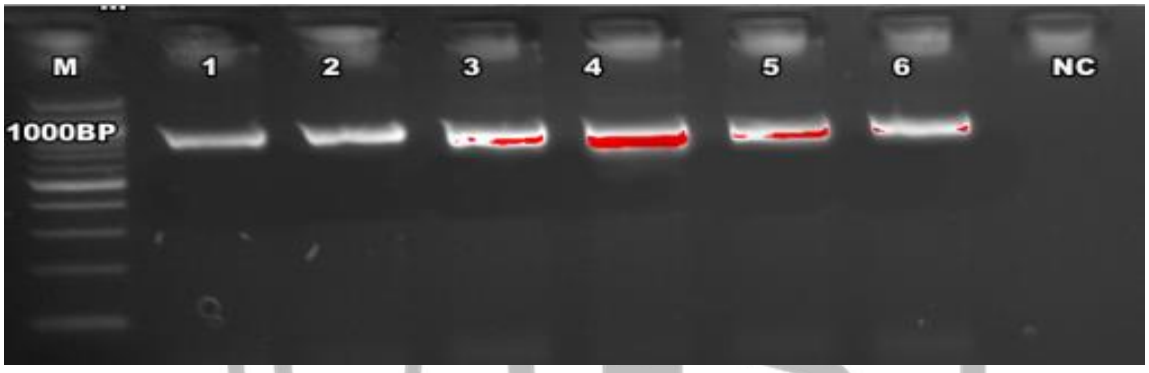


Fig 3: PCR detection of TEM gene (840 bp) PCR visualized under gel documentation. Lanes 1,2,3,4,5 and 6 are positive. NC is negative control. M is marker (100bp).

**Virulence genes profiling**

A total of 10 different types of virulence genes occurred amongst the 8 ESBL isolates. In a, 31 of these genes occurred in different prevalence. Amongst the iron acquisition virulence genes, *Chu A*(fig 4)*, iro N, Iuc D and Sit Ep* occurred at 25%, 12.5%, 25% and 25% respectively. Serum resistance were represented by *Iss, ompA and Tra T*(fig 4) and they occurred in 75%, 25% and 62.5% respectively amongst the ESBL isolates (fig 5). Ahesin, Toxin and Invasins were represented by *Tsh, Ast A* and *Ibe A* which occurred in 12.5%, 62.5% and 62.5% respectively. Iron acquisition and serum resistance genes were significant (Table 2).

**Table 2: prevalence of virulence genes amongst ESBL isolates**

| Virulence factor | No of ESBL isolates harboring genes n=8 | percentage | p-value |
|---|---|---|---|
| **Iron acquisition** | | | |

| | | | |
|---|---|---|---|
| *Chu A* | 2 | 25 | |
| *Iro N* | 1 | 12.5 | |
| *Iuc D* | 2 | 25 | |
| *Sit Ep* | 2 | 25 | 0.05 |
| **Serum resistance** | | | |
| *Iss* | 6 | 75 | |
| *Omp A* | 2 | 25 | |
| *Tra T* | 5 | 62.5 | 0.01 |
| **Adhesin** | | | |
| *Tsh* | 1 | 12.5 | --- |
| **Toxin** | | | |
| *Ast A* | 5 | 62.5 | --- |
| **Invasin** | | | |
| *IbeA* | 5 | 62.5 | --- |



Amongst the 31 virulence genes detected, genes responsible for serum resistance genes occurred in the highest number of ESBL isolates as compared to the other four categories of virulence genes. These genes were harbored in all ESBL samples (100%). Adhesins were harbored by 12.5% of the samples while invasins, iron acquisition and toxins were each harbored by 62.5% of the total number of samples.

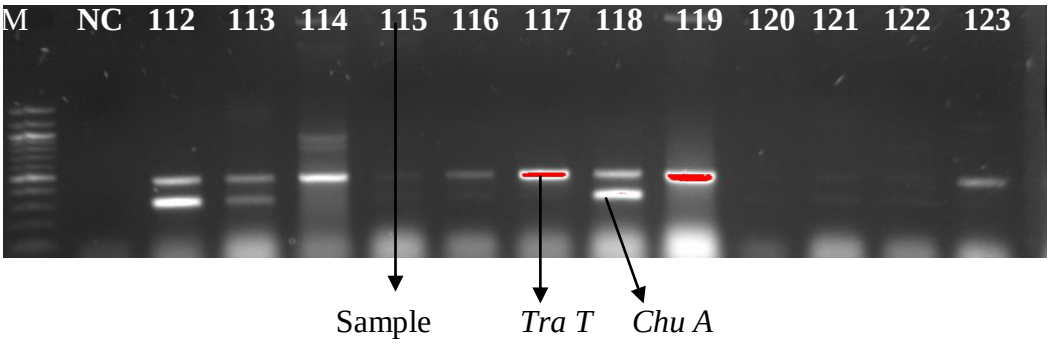


**Figure 4:** PCR detection of virulence of APEC( *traT and chuA*). Multiplex PCR visualized under gel documentation. Lanes 112,113,114,116,117,118119 and 123 are positive for gene *tra T* (430bp) whiles lane 112,113 and 118 are positive for gene *chuA*(278bp). NC is negative control. M is marker (100bp).

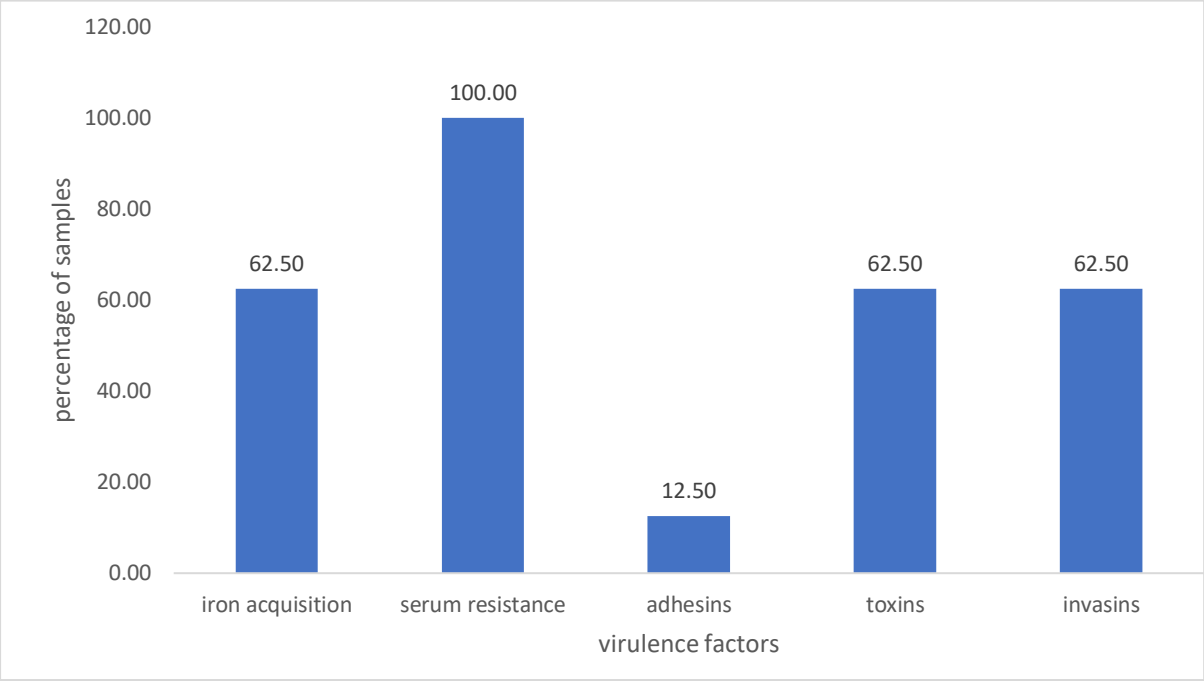


**Fig 5: occurrence of virulence genes amongst ESBL isolate**

Table 3 – Relationship of virulence genes and their combinations inESBL isolates

| Genes | No of samples (%) |
|---|---|
| *Iss* | 6 (75%) |
| *tra T* | 5 (62.5%) |
| *Ast A* | 5 (62.5%) |
| *Ibe A* | 5 (62.5%) |
| *Iss + ibeA* | 4 (50%) |
| *Tra T + Ast A* | 4 (50%) |
| *Iss + tra T* | 3 (37.5%) |
| *Iss + ast A* | 3 (37.5%) |
| *Ibe A + Ast A* | 3 (37.5%) |
| *Iss +ibeA + Ast A* | 2 (25%) |
| *Tra T+ Ast A + Ibe A* | 2 (25%) |
| *Chu A + Ibe A + iss* | 2 (25%) |
| *Iucd+ iss + ibeA* | 2 (25%) |
| *Sit Ep + omp A + tra T* | 2 (25%) |



Two dual categories of the genes were harbored by 50% of the samples; these are *iss/ibeA* and *tra T/Ast A*. The other dual combinations were each harbored by 37.5% of the ESBL isolates (*iss/tra t, iss/ast A* and *ibeA/ast A*). Tree combinations of genes were also harbored by 25% of the ESBL isolates, these include: *iss/ibeA/AstA, tra T/AstA/ibeA, ChuA/ibeA/iss, iucD/iss/ibeA and sitEp/ompA/tra T*. (Table 3)

**Table 4: Association of Virulence Factors and Resistance Genes**

| | Chu A | Iro N | Iuc D | Sit Ep | Iss | Omp A | Tra T | Tsh | Ast A | Ibe A | TEM | MA | CTXM-1 | CTXM-825 | CTXM-914 | CMY | OXA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Chu A* | NA | | | | | | | | | | | | | | | | |
| *Iro N* | 0.488 | NA | | | | | | | | | | | | | | | |
| *Iuc D* | 0.149 | -0.218 | NA | | | | | | | | | | | | | | |
| *Sit Ep* | 0.149 | -0.218 | -0.333 | NA | | | | | | | | | | | | | |
| *Iss* | 0.447 | 0.218 | 0.333 | -0.333 | NA | | | | | | | | | | | | |
| *Omp A* | 0.149 | -0.218 | -0.333 | 1.000 | -0.333 | NA | | | | | | | | | | | |
| *Tra T* | -0.775 | -0.378 | -0.577 | 0.000 | -0.577 | 0 | NA | | | | | | | | | | |
| *Tsh* | -0.293 | -0.143 | -0.218 | -0.218 | -0.655 | -0.218 | 0.378 | NA | | | | | | | | | |
| *Ast A* | -0.467 | -0.488 | -0.149 | -0.149 | -0.447 | -0.149 | 0.775 | 0.293 | NA | | | | | | | | |
| *Ibe A* | 0.067 | 0.293 | 0.447 | -0.745 | 0.149 | -0.745 | -0.258 | 0.293 | -0.067 | NA | | | | | | | |
| *TEM* | | | | | | | | | | | NA | | | | | | |
| *MA* | 0.258 | 0.378 | 0.000 | -0.577 | 0.000 | -0.577 | 0.000 | 0.378 | 0.258 | 0.775 | | NA | | | | | |
| *CTXM-1* | -0.293 | -0.143 | -0.218 | -0.218 | 0.218 | -0.218 | 0.378 | -0.143 | 0.293 | 0.293 | | 0.378 | NA | | | | |
| *CTXM-825* | -0.258 | 0.378 | -0.577 | 0.000 | -0.577 | 0.000 | 0.500 | 0.378 | 0.258 | -0.258 | | 0.000 | -0.378 | NA | | | |
| *CTXM-914* | -0.067 | 0.488 | -0.447 | 0.149 | -0.149 | 0.149 | 0.258 | -0.293 | 0.067 | -0.467 | | -0.258 | -0.293 | 0.775 | NA | | |
| *CMY* | -0.258 | 0.378 | -0.577 | 0.000 | -0.577 | 0.000 | 0.500 | 0.378 | 0.258 | -0.258 | | 0.000 | -0.378 | 1.000 | 0.775 | NA | |
| *OXA* | 0.447 | 0.218 | -0.333 | 0.333 | -0.333 | 0.333 | 0.000 | 0.218 | 0.149 | -0.447 | | 0.000 | -0.655 | 0.577 | 0.447 | 0.577 | NA |

The MA gene showed positive correlation with *ChuA, iroN, Tsh Asta and IbeA* but negative correlation with *Sit Ep*, and *Omp A* and no association with *IuCD, Iss and Tra T*. CTXM-1 gene recorded a positive correlation with *iss, tra T, astA and ibeA*. CTXM-825 had positive correlation with *iroN, tra T, tsh* and *ast A*. CTXM-914 also showed positive correlation with *iroN, sit EP, OmpA, , tra T and Ast A*. CMY also recorded a positive correlation with *Iro N, tra t, tsh and ast A* whiles OXA showed a positive correlation with all samples except *iucD, iss, tra t and ibe A* (Table 4).

**Table 5: prevalence of virulence genes amongst housing system and site of infection**

| Category | | No of virulence genes n=31 | P-value |
|---|---|---|---|
| Housing system | Extensive | 13 | 0.309 |
| | Semi-Intensive | 18 | |
| Site of infection | Oral-pharyngeal | 20 | <0.05 |
| | Cloacal | 11 | |

The number of virulence factors recorded varied from housing systems and site of infection. The isolates from the extensive system recorded a total of 13 as against 18 of the semi-intensive system whiles oral-pharyngeal recorded 20 virulence genes as opposed to 11 of the cloacal (Table 5)

**DISCUSSION**

To the best of our knowledge, this is the first time virulence genes distribution has been described amongst ESBL isolates in scavenging local chicken in Tanzania. Studies involving virulence genes and resistance genes in chicken have always focused on broilers and layers (21, 45) One of the primary control measures to reduce morbidity and mortality caused by APEC is Antimicrobial therapy (2). The frequent use of antimicrobials has however allowed the selection of resistant isolates and this has become a global health concern (37). The presence of these virulence genes in *E. coli* may cause disease in scavenging chicken and thereby pose a public health risk through the food chain, transferring resistance genes to potential human pathogens.



The study observed that the highest and significant resistant was to the nalidixic acid drug (87.5%). A study by Johnson *et al.,* 2003 (23), indicate that Nal-resistant *E. coli* is prevalent in poultry and that a substantial amount of such strains represent potential human pathogens. Sparing resistances were recorded against drugs CTX (cefotaxime), STX (trimethoprim-sulfamethoxazole), AUG (augmentin), and KF (ciprofloxacin). Of the total samples, 37.5%, were resistant against these antibiotics. According to (5), these drugs are first choice for the treatment of human patients and thus the trend of resistance noted in this study is quiet alarming.

Our study observed a significant ESBL phenotypes amongst the APEC isolates ,42% (8/19). This confirms that ESBL production is a critical resistance mechanism to antimicrobials (14). Earlier studies recorded higher percentages; Kanaba et al., 2019 (24) recorded 72.1% of ESBL phenotype amongst isolates whiles Casella(6) observed 96.1% (74/77) positive for ESBL in *E. coli* isolates from poultry in France. Both of these studies detected the ESBL isolates directly from amongst *E.coli*

isolates, whiles in this study APEC samples were first detected from the *E.coli* and then the ESBL isolates detected from APEC.

The most prevalent ESBL gene was TEM, it was detected is all ESBL samples. CMY-2 was found amongst 62.5% whiles the CTX-M had varying percentages; (CTX-M 825 50%, CTXM-914 37.5% and CTXM-1 12.5%). Data produced in this study are in consonance with the most commonly described ESBLs and AmpC producing *E. coli* in poultry production; CTX-M, TEM, CMY-2 and SHV (9,17).

The rampant occurrence of these genes with with a high virulence potential has been observed in human infections as well (10, 4). What is noteworthy that these are isolated from scavenging chicken and hence promotes zoonotic occurrence since they share same environment as human. What is also noteworthy is the presence of an *E. coli* isolate producing CMY-2 and their co-resistance to flouroquinolones (qnrS). This phenomenon is rare and was first detected by Sola-Grines *et al.,*2015 (47). All of these isolates were resistant to flouroquinones including nalidixic acid.

Forty percent of the the virulence genes detected amongst the ESBL isolates were with a frequency higher than 50% . This discrepancy in prevalence has shown that the ESBL isolates have high variability. It also proves the heterogeneity of the APEC isolates at noted by Lopez *et al.,* 2017(32).The most prevalent virulence include *iss*, *tra T, Asta A* and *ibe A*. Whiles iss was harbored by 75% of the samples*, tra T, Asta A* and *ibe A* were each harbored by 62.5% of the isolates. These genes are noted to be associated with plasmids and regarded as good APEC markers. They are also correlated with disease severity (46). Similar results were reported by Hiki *et al.,* 2014(19). Ozaki *et al.,* 2017 (39) also noted that the iss gene was detected in 93% of APEC isolates, which was the highest. Although we also had the iss gene being the highest, we detected them amongst 75% of isolates whiles they detected them amongst 75%. The difference was because they detected the virulence genes amongst APEC directly whiles we detected them amongst ESBL isolates. Both *Tra t* and *iss* belong to the serum resistance group of virulence genes, together with the *Omp A* gene. These outer membrane proteins that are synthesized to allow the APEC to invade host defenses (49). All ESBL isolates harbored at least one of the genes in this group. *E. coli* with serum resistance are noted to be highly virulent and can escape the complement system and promote serum survival.

This can increase the risk of developing septic shock and the increase in mortality (33,34). The only ESBL gene that recorded a positive correlation with *iss* was the CTX-M1 gene, which was recorded amongst the least number of samples, less than 20%. Not surprisingly this positive correlation was not strong and not significant. This is an implication that the occurrence of the iss gene is independent on any of the ESBL genes. A different observation was made with regards to the *tra T* gene. Apart from the MA and OXA gene, the *tra T* gene recorded a positive correlation with all the other ESBL genes; CTX-M1, CTX-M825 and CTX-M945 and CMY genes with more significant and stronger positive correlation with CTX-825 and CMY genes. Several reports both in veterinary and human medicine has made same observations.Cergole-Novella (8) showed that there was an association between *traT* and with bla−CTX-M in *E. coli* isolated from gastroenteritis from humans. Simillar conclusion was drawn by El-Baky *et al.,* 2020 (15). The *IbeA* virulence gene showed positive correlation to only MA and CTXM-1. This correlation was stronger in MA than in CTXM-1. *Ast A* is the only virulence gene that had positive correlation with all the ESBL genes; all of which were moderate correlation except CTXM-914 which had a low correlation.

Four of the 10 detected virulence genes are responsible for iron acquisition; *ChuA, iroN , iucD*, and *sit Ep*. Together, these are harbored by 62.5% of ESBL isolates. According to Joeng *et al*., 2012, there is redundant iron uptake systems in APEC and gives it the ability to function in different host niches. These genes are responsible for iron uptake transporting ferrous iron and considered the main virulence factors of APEC (28)

Earlier report has describe the *Tsh* gene as having an adhesion capacity to Caco-2 cells (18), and its varying prevalence between 10% to 90% has been reported by several studies (43, 26, 27,40). In this study, it was detected in 12.5% of ESBL strains, which is in close conformity to study by Rodriquez-Siek et al., 2005 (43). None of virulence genes of *Chu A, IucD*, *sit Ep* and *Ompa* we iroN, and tsh showed significant positive correlation with any of the ESBL genes.

A number of genes and their combinations increases the virulence of APEC, making it mutagenic. It is therefore critical to consider these combinations in defining the molecular pathotypes of APEC. Some of these combinations are indications of mobile genetic elements like pathogenic islands (PAIs)

and plasmid. The highest dual combination included *iss/ibeA*, and *traT/Asst A*. These are harbored by 40% of ESBL isolates. Another combination of interest is that of *iss/iroN*, although that occurred in only 12.5% of ESBL isolates. All these combinations are indicative of non-colV plasmid. The difference in this prevalence could be due to the fact that the *iroN* gene is found in conserved regions whiles the others are in the APEC whiles the others are found in variable regions variable region making highly mobile.

The number of virulence genes in the oral pharynges of the chicken were significantly higher than those of the cloacae of the chicken. Since this poultry scavenge mainly for their feed, the conclusion is that one of the main source of the virulence genes they harbored could be from the environment. And because they shared this same environment with humans, these genes has high zoonotic potential. It would therefore be needful to carry out same research on screen environmental samples. This is also an indication that seemingly healthy poultry could harbor virulence genes, since the number of genes they release to back to the environment (through their cloacal) is less than the number they pick from it(oral-pharyngeal).de Oliveira *et al.,* 2020 (38) reported on the existence of virulence genes in poultry that does not have history of cellulitis.



**CONCLUSION**

The study observed that majority of ESBL isolates offered the highest and most significant resistance to the nalidixic acid drug (87.5%) and sparing resistances to drugs CTX (cefotaxime), STX (trimethoprim-sulfamethoxazole), AUG (augmentin), and KF (ciprofloxacin). The most prevalent virulence include found amongst ESBL isolates include *iss*, *tra T, Asta A* and *ibe A*. The *tra T* virulence gene recorded a positive correlation with ESBL genes CTX-M1, CTX-M825 and CTX-M945 and CMY whiles the *IbeA* virulence gene showed positive correlation to only MA and CTXM-1. Scavenging local chick tend to harbor more virulence genes in them as they release a lesser amount to the environment compared to the number the take from there. The relationship between virulence factors and resistance genes remains is complex and requires further investigation.

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