# Antibiotic Resistance and Plasmid Analysis of Enterobacteriaceae Isolated From Retail Meat in Lagos Nigeria

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**Research**

**Keywords:** Enterobacteriaceae, plasmids, antibiotic, resistance

**Posted Date:** December 16th, 2020

**DOI:** https://doi.org/10.21203/rs.3.rs-126037/v1

## Abstract

**Background** The presence of antibiotic resistant microorganisms in food is of great concern globally. This research was carried out to detect and characterize plasmid carriage and profiles among members of Enterobacteriaceae from different meat types in Nigeria.

**Method** From a total of 80 meat samples comprising of mutton, pork, beef and chicken, organisms belonging to the family Enterobacteriaceae were isolated by standard procedures and identified by API 20E system. Antibiotics susceptibilities testing (AST) against selected classes of antimicrobial agents and plasmid extraction was carried out by disc diffusion and alkaline lysis methods respectively.

**Results** One-hundred and ten Enterobacteriaceae were isolated, species identification revealed isolates belonging to 7 genera comprising *of Escherichia*, *Enterobacter*, *Klebsiella*, *Citrobacter, Proteus*, *Salmonella* and *Serratia.* Overall resistance of the organisms to amoxycillin/clavulanic acid was 91 (82.7%), streptomycin 85 (75.7%) and perfloxacin 74 (67.2%) while ofloxacin had the highest susceptibility rate (91.8%). Plasmids profiling revealed ranges of plasmids from 1 to 3 copies with estimated sizes range of 700bp to 1.1kb among *E. coli*, *K. pneumoniae*, *E. aerogenes* and *Proteus mirabilis*. All the isolates with plasmids were multidrug resistant and were isolated from chicken except a strain of *E. coli* from pork which harboured a single plasmid copy suggesting these meat as reservoirs for antibiotic resistant bacteria.

**Conclusion** Our findings revealed high level of meat contamination with antibiotic resistant Enterobacteriaceae harbouring resistant plasmids. An integrated surveillance system and safety practice must be ensured among the processors and retailers


## Introduction

Foodborne diseases associated with contaminated meats and meat products is a major public health issue. Inadequate food handling practices such as poor sanitation exercise, weak or poor safety laws and regulatory system enforcements, lack of enlightenment and infection awareness are some of the major factors promoting foodborne diseases in developing countries [1]. The microbiological quality of any meat depends on the health and physical status of the animal at the point of slaughter, handling, environmental hygiene and storage [2].

The family Enterobacteriaceae consists of a large heterogeneous group of Gram-negative rod-shaped bacteria that are naturally found in the mammalian gut although can also be found in other environments. They are useful indicators of food quality, hygiene and contamination, examples include *Escherichia coli, Salmonella* spp. and *Shigella* spp. Enterobacteriaceae is responsible for a range of enteric infections such as diarrhea and endocarditis, to infections of the respiratory tract, skin, soft-tissues, urinary tract, joints, bones, eyes and central nervous system [3].

Contamination of meat and meat products by antibiotic-resistant bacteria is of serious concerns and a major risk to public health. Of particular interest is the effect of contamination by antibiotic-resistant members of the family Enterobacteriaceae, particularly *E. coli*, *Salmonella* spp., which are few amongst the top foodborne pathogens of global relevance. Food outbreaks of significant impacts with high death tolls have been attributed to members of this family in the past, especially *E. coli* and *Salmonella* Spp. Reports of elevated antibiotic resistance and dissemination among these foodborne pathogens have also been previously reported [4,5].

Antibiotic use in livestock production is sometimes unavoidable because of the treatment of infections caused by bacteria or other microbes of which therapies or vaccines are not readily available. It is a fact that these antibiotics are sometimes given at a sub-therapeutic dosage and this often leads to selective pressure and proliferation of antimicrobial resistance and spread among the animal intestinal flora. *E. coli* and other members of Enterobacteriaceae have been described with high proficiency for transmission of antibiotic resistance genes via mobile genetic elements such as plasmids and integrons to other intestinal organisms [6].

Meat is consumed in many homes in Nigeria. It is a nutrient-rich food with a vital amount of proteins, vitamins and minerals as well as great bioavailability than other food sources [7]. The rate of consumption of meat in Nigeria is at a high rate even though the meat has been recognized as one of the main vehicles for the transmission of foodborne pathogens to human [8]. Most foodborne outbreaks are associated with eating of contaminated meats and meats products, and that the occurrence of members of Enterobacteriaceae in animal-derived products is high, then it is imperative to always carry out research based on the identification of the genera involved and to assess their level of contaminations and danger they pose to public health. This will be expected to provide valuable data needed for the logical assessment of outbreaks and disease infections associated with foodborne pathogens as well as to elucidate on the role of antibiotic resistance transmission from the farm to the table.

The aim of the present study was to detect members of Enterobacteriaceae in locally processed meat samples and to investigate the presence of plasmids as a mode of antibiotic resistance gene transmission among them.

## Materials And Methods

### Sample collection

A cross-sectional study was conducted in processed retail meat to determine the bacteriological quality and antibiotic susceptibility of Enterobacteriaceae in Lagos metropolis. A total of 80 samples of retail meat [comprising of mutton (*n*=20), pork (*n*=20), beef (*n*=20) and chicken

(*n*=20)] were randomly purchased from retail points at Agege, Obalende, Mushin and Bariga in Lagos Metropolis from March to June 2019. The meat samples were collected in ziplock bags and immediately transported to the laboratory for microbiological analysis.

### Sample analysis

All meat samples were assayed for the presence of any member of Enterobacteriaceae by weighing 25g of each meat sample aseptically and added to 225 ml of sterile 0.1% buffered peptone water and blended for 2 min. in sterile stomacher bag [9]. Tenfold serial dilutions of up to $10^6$ were made from the homogenized sample and 1ml from each final dilution was plated on Petri dishes containing different agars of MacConkey, Eosin Methylene Blue, *Salmonella-Shigella* and incubated for minimum of 24 h until visible growths were observed. Isolates were subcultured based on their phenotypic appearances and colonial morphologies, for instance isolates that appeared on MacConkey agar (MCA) as lactose and non-lactose fermenters, were subculture separately on different MCA and Salmonella-Shigella agar (SSA). Colonies that appeared as dark centered colonies and those with green metallic sheen were picked and subculture on SSA and Eosin methylene blue agar (EMBA) respectively and subsequently screened on sorbitol MacConkey agar (SMAC) as described [9]

### Identification of isolates

All pure cultures of suspected members of Enterobacteriaceae were subjected to preliminary standard biochemical test for the identification. Presumptively identified members of Enterobacteriaceae were further screened by using API 20E system (Bio-Merieux, France) according to manufacturer's instructions.

### Antimicrobial susceptibility testing

The antibiotics susceptibility of the isolates against commonly prescribed drugs for enteric and foodborne infections was determined and interpreted by standard procedures and guidelines for disc diffusion method [10]. The following antibiotics were used; trimethoprim/sulfamethoxazole (25µg), chloramphenicol (30µg), ciprofloxacin (10µg), amoxicillin (30µg), amoxicillin/clavulanic acid (20/10µg), gentamicin (10µg), pefloxacin (30µg), ofloxacin (10µg), streptomycin (30µg).

### Plasmid DNA extraction

The plasmid extraction was carried out as previously described [11] with little modifications using 100bp and 1kb plasmid ladders. Twenty-five (*n*=25) isolates were picked at random among the antibiotics resistant isolates for the purpose of plasmid investigation. Briefly, bacterial cultures were grown in nutrient broth with an optimized concentration of antibiotics at 37 °C overnight in a shaker incubator at 150 rpm. After harvesting, the culture was transferred to a 1.5-ml Eppendorf tube containing a lysis buffer, heated for 15 min at 70$^{o}$C, mixed with an equal volume of phenol: chloroform: isopropanol (25:24:1) and afterwards centrifuged. The supernatants were collected using pipette, and added to a blue loading dye and run on 1% agarose gel Tris–acetate–EDTA buffer for an hour at 80 V. plasmid bands were visualized using Gel Documentation system and ultraviolet light transilluminator.

### Statistical analysis

A one-way analysis of variance (ANOVA) test was used to compare the difference in the prevalence of isolates recovered from the various categories of samples with significant level at $p < 0.05$

## Results

A total of 110 isolates of members of Enterobacteriaceae were obtained from the 80 meat samples analyzed. The colonial morphology, Gram reaction and results obtained from the API 20E kits revealed the isolates identity as *Enterobacter aerogenes, E. cloacae, Citrobacter freundii, Proteus mirabilis, Klebsiella pneumonia, K. planticola, Salmonella* spp., *E. coli*, and *Serratia odorifera.* The frequency of occurrence of these organisms in the meat samples is as shown in Table 1 with *Enterobacter* spp. having the highest frequency of occurrence of 26 (23.6%) and *Serratia* spp. having the lowest 3 (2.7%).

Table 1
Frequency of occurrence of members of Enterobacteriaceae in locally processed meat

| Enterobacteriaceae | Beef | Pork | Chicken | Mutton | Total Frequency (%) |
|---|---|---|---|---|---|
| Proteus **spp.** | 5 | 2 | 0 | 4 | 11 (10) |
| Enterobacter **spp.** | 9 | 13 | 2 | 2 | 26 (23.6) |
| Citrobacter **spp**. | 2 | 0 | 7 | 5 | 14 (12.7) |
| Escherichia coli | 8 | 9 | 4 | 2 | 23 (21) |
| Serratia **spp**. | 0 | 2 | 1 | 0 | 3 (2.7) |
| Klebsiella **spp**. | 5 | 7 | 4 | 2 | 18 (16.4) |
| Salmonella **spp**. | 0 | 5 | 5 | 5 | 15 (13.6) |
| **Grand Total** | 29 | 38 | 23 | 20 | 110 (100) |
| **Standard Deviations** | 3.6253 | 4.5774 | 2.43 | 1.8645 | 3.6253 |

# Antibiotics Susceptibility Test

The antibiotics resistance profile of the organisms is presented in Table 2. Ninety-one (82.7%) of Enterobacteriaceae isolated were resistant to amoxicillin/clavulanic acid while 85 (75.7%) were resistant to both amoxicillin and streptomycin, 74 (67.2%) were resistant to perfloxacin while 65 (59.1%) showed resistance to both sparfloxacin and ciprofloxacin. Similar resistance level of 45 (40.9%) was found for both gentamicin and trimethoprim/sulfamethoxazole while 91.8% of the organisms were susceptible to ofloxacin.

Table 2
Resistance of enteric bacteria to antibiotics.

| Enterobacteriaceae | Number of resistant strains (Percentage Resistance) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total No. | AMX | AUG | GEN | CHL | SPX | TIM | CIP | OFX | STR | PEF |
| *Proteus* spp. | 11 | 11(100) | 9(81.8) | 10(90.9) | 10(90.9) | 7(63.6) | 9(81.8) | 6(54.5) | 0(0.0) | 11(100) | 10(90.9) |
| *Enterobacter* spp. | 26 | 14(53.8) | 26(100) | 15(57.7) | 0(0.0) | 13(50) | 0(0.0) | 15(58.8) | 0(0.0) | 7(26.9) | 20(76.9) |
| *Citrobacter* spp. | 14 | 7(50) | 13(92.9) | 6(42.9) | 12(85.7) | 8(57.1) | 0(0.0) | 9(64.3) | 7(50) | 14(100) | 5(35.7) |
| *Serratia* spp. | 3 | 3(100) | 3(100) | 1(33.3) | 0(0.0) | 0(0.0) | 2(66.7) | 0(0.0) | 0(0.0) | 0(0.0) | 1(33.3) |
| *Klebsiella* spp. | 18 | 15(83.3) | 2(11.1) | 3(16.7) | 1(5.6) | 15(83.3) | 11(61.1) | 17(94.4) | 0(0.0) | 16(88.8) | 18(100) |
| *Salmonella* spp. | 15 | 12(80) | 15(100) | 10(83.3) | 12(80) | 11(61.1) | 0(0.0) | 10(55.5) | 0(0.0) | 14(93.3) | 10(55.5) |
| *E. coli* | 23 | 23 (100) | 23 (100) | 0 | 13(37.5) | 8(56.5) | 23(100) | 2 (8.6) | 2(8.6) | 23(100) | 5 (21.7) |
| Total | 110 | 85(77.2) | 91(82.7) | 45(40.9) | 48(43.6) | 65(59.1) | 45(40.9) | 65(59.1) | 9 (8.18) | 85(77.3) | 74(67.2) |

AM: Amoxicillin (30ug), AMC: Amoxycilin/clavulanic acid (20/10ug), GEN: Gentamicin (10ug), CHL: Chloramphenicol (30ug), SPX: Sparfloxacin (10ug), TIM: trimethoprim/sulfamethoxazole (30ug), CIP: Ciprofloxacin (10ug), OFX: ofloxacin (10ug), STR: Streptomycin (10ug), PEF: Pefloxacin (30ug).

At genera level, complete resistance (100%) was observed among *Proteus* spp. against amoxicillin and streptomycin, *Enterobacter* spp. showed complete resistance to amoxicillin/clavulanic acid, so were *E. coli*, *Serratia* spp. and *Salmonella* spp. There was 100% resistance by *E. coli* to trimethoprim/sulfamethoxazole, amoxicillin and streptomycin. *Klebsiella* spp. and *Citrobacter* spp. showed complete resistance to perfloxacin and streptomycin respectively. In terms of susceptibilities, there was 100% susceptibility to chloramphenicol, sparfloxacin, ciprofloxacin, ofloxacin and streptomycin by *Serratia* spp. *Enterobacter* spp. were completely susceptible to chloramphenicol, ofloxacin and trimethoprim/sulfamethoxazole, while *E. coli* and *Salmonella* spp. were completely susceptible to gentamicin, trimethoprim/sulfamethoxazole and ofloxacin respectively.

# Plasmid Analysis

Nine (36%) out of 25 isolates harboured plasmids ranging from 1 to 3 copies with estimated size range of 700 bp to 1.1 kb and highest number of plasmids 4 (44.44%) was detected in *E. coli*, followed by *K. pneumonia* and *E. aerogenes* both having 2 (22.22%) each, and *P. mirabilis* 1 (11.11%).

Maximum of 3 copies of plasmids was found in *E. coli* isolated from chicken while remaining isolates had 1 copy each. All the isolates with plasmids were from chicken except a strain of *E. coli* from pork harboring a single plasmid copy.

# Statistical Analysis

According to the statistical analysis, the *f*-ratio value is 0.82804 while the *p*-value is 0.491442 hence there was no significant difference in the incidence of Enterobacteriaceae in pork, beef, mutton and chicken at $p < 0.05$ significant level.

## Discussion

In this study, Enterobacteriaceae were isolated from locally processed beef, pork, mutton and chicken on retail. In overall, there was no significant difference in Enterobacteriaceae contamination levels among the different meat types, however highest number of isolates 38 (34.5%) recovered was from pork and *Enterobacter* spp. dominated the overall population of the isolates recovered as 23% of its species were isolated from different meat samples investigated in this study followed by *E. coli* 21% while *Serratia* spp. was the least isolated from all the meat (Table 1).

Since the presence of Enterobacteriaceae is an indicator of hygiene and post processing contamination of food products, contamination of retail meats by Enterobacteriaceae in the present study suggests a possible breakdown in hygienic conditions and safety practices. Previous studies have documented the presence of one or more of members of Enterobacteriaceae from retail meat and carcasses of animals. Uzeh and Agunlanna [12] detected *E. coli* O157:H7 and other *E. coli* strains in 37% and 63% respectively of meat samples from different parts of cattle carcass. In consistent with the results of this study, although at varying prevalence, Enterobacteriaceae have been previously reported from different retail meat samples. Studies from Egypt documented the presence of *Proteus* spp., *E. coli Citrobacter* spp. and *Klebsiella* spp. in retail meats from sellers [13], *Klebsiella* spp., *Serratia* spp., *E. coli*, *Enterobacter* spp., *Citrobacter* spp., *Proteus mirabilis* and *Serratia* spp., were also reported from retail beef and poultry in the United State [14].

Enterobacteriaceae isolated in this study showed high level and multiple resistance traits to antibiotics investigated, which can be transmitted from food products to human via consumption. Out of all the antibiotics investigated for activities against these pathogens in this study, ofloxacin remained the most potent with an overall susceptibility rate of 91.8% by the organisms. Compared to other members of fluoroquinolones investigated in this present study, ofloxacin showed remarkable higher efficacy than ciprofloxacin and sparfloxacin both with equal overall 40.9% susceptibility rate, and perfloxacin with 32.8% susceptibility rates. Ofloxacin higher antimicrobial activities has been attributed to the presence of alkylated piperazine group at one of its structural positions [15].

Diverse rates of resistance to the different classes of antibiotics was observed in this study and it ranged from 5.6–100% with resistance to streptomycin, trimethoprim/sulfamethoxazole, amoxicillin and amoxicillin/clavulanic acid being most frequently observed. The observed resistance to antibiotics in this study is so disturbing considering the facts that most of the antibiotics investigated are not commonly used in the livestock production but are rather prescribed against human's infections caused by enteric bacteria. These results are in agreement with previous related studies that reported similar high resistance to the above antimicrobial agents [16]. A high level of resistance or complete resistance (100%) to amoxicillin and amoxicillin/clavulanic acid was observed by 85% of the isolates in this study. Both drugs belong to beta-lactam and beta-lactamase inhibitor classes of antibiotics respectively, and are commonly prescribed for infections associated with Gram negative bacteria. Resistance to these two classes of antibiotics is usually mediated by beta-lactamase enzymes encoded on genes borne on mobile genetic elements such as plasmids and integrons that enables them to efficiently hydrolyze these drugs [17, 18].

Enterobacteriaceae are well known for harbouring plasmids in multiple copies of varying sizes [19]. The occurrence of plasmids among the resistant Enterobacteriaceae in this study was detected among 9 out of 25 isolates investigated, accounting for 36% of the total Enterobacteriaceae investigated for plasmids. Isolates positive for the presence of plasmid harboured up to 3 copies of the extra-chromosomal DNA with estimated sizes of up to 1.1 kb. Carriage of plasmid among the selected isolates was more frequent among bacteria isolated from chicken while *E. coli* had the highest number of prevalence among the investigated isolates with plasmids. The findings from the present study is in agreement with previous studies on carriage of plasmids by members of Enterobacteriaceae [19, 20]. There was a correlation between plasmid profiles of the isolated bacteria and their multiple drug resistance in this study (Table 3). Carriage of plasmids among resistant Enterobacteriaceae in the present study suggests the resistance profile demonstrated by the isolates might be associated with the presence of one or more resistant mechanisms encoded on the plasmids. Plasmids often carry genes encoding multiple resistance to drug classes such as fluoroquinolones, aminoglycosides and are capable of transmission by horizontal gene transfer among bacterial communities [21, 22]

Table 3
Plasmid Profiles of 9 Enterobacteriaceae isolated from various meat sources

| Source | Isolates | Plasmid sizes (kb) | Plasmid copies | Resistance profile |
|---|---|---|---|---|
| Chicken | *E.coli* BCI15 | 0.7, 0.75, 1.1 | 3 | AMX, AUG, CHL, SPX, TIM, CIP, OFX, STR, PEF |
| Chicken | *K. pneumoniae* BCI28 | 0.7, 0.9 | 2 | AMX, AUG, CHL, SPX, TIM, CIP, STR, PEF |
| Chicken | *K. pneumoniae* ACI77 | 0.75, 0.9 | 2 | AMX, AUG, GEN, SPX, TIM, CIP, STR, PEF |
| Chicken | *E. aerogenes* OCI46 | 0.7, 0.8 | 2 | AMX, AUG, GEN, SPX, CIP, STR, PEF |
| Chicken | *E. aerogenes* ACI12 | 0.7, 0.75 | 2 | AMX, AUG, GEN, SPX, CIP, STR, PEF |
| Chicken | *E.coli* ACI22 | 0.8 | 1 | AMX, AUG, CHL, SPX, TIM, CIP, STR, PEF |
| Chicken | *E.coli* OCI50 | 0.8 | 1 | AMX, AUG, CHL, SPX, TIM, STR, PEF |
| Chicken | *P. mirabilis* BCI08 | 0.7 | 1 | AMX, AUG, GEN, CHL, SPX,TIM, CIP, STR, PEF |
| Pork | *E.coli* API33 | 0.75 | 1 | AMX, AUG, CHL, SPX, TIM, STR, PEF |

In conclusion, the data presented in this study shows that locally processed pork, beef, mutton and chicken on retail may contribute significantly to the spread of antibiotic-resistant Enterobacteriaceae to the community. High prevalence of antibiotic resistant Enterobacteriaceae in these retail meats is a potential risk to public health. It is also clear from this study that certain levels of safety practices that help to minimize food contaminations may have been compromised in the course of meat processing and during retail. Measures should therefore be put in place to reduce the proliferation of pathogenic antibiotic resistant bacteria in these meat products.

## Abbreviations

AST
Antibiotics susceptibilities testing, API 2:Analytical Profile Index, MCA:MacConkey agar, SSA:Salmonella-Shigella agar, EMBA:Eosin methylene blue agar, ANOVA:one-way analysis of variance

## Declarations


### Acknowledgments

The authors would like to appreciate the technical staff of the Department of Microbiology, University of Lagos for their support during the course of the experimental work


### Authors contributions

REU designed and conceptualized the work, sampling and experimental work was carried out by FA under the guidance of REU. The data was analyzed and interpreted by BTO, manuscript was drafted by BTO and was revised critically by REU. All authors read and approved the manuscript


### Funding

The author (s) did not receive any specific funding for this work


### Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request. All data generated or analyzed during this study are also included in this published article

### Ethics approval and consent to participate

The work was exempted from ethical approval since the samples used were purchase from retailers

### Consent for publication

Not applicable

### Competing interests

None to declare

## References


1. World Health Organization (WHO). Developing and maintaining food safety control systems for Africa: current status and prospects for change. InSecond FAO/WHO Global Forum of Food Safety Regulators; Oct 12–14, 2004; Bangkok, Thailand 2004 Oct 12.
2. Ilboudo JA, Tapsoba F, Savadogo A, Seydi M, Traore AS. Improvement of the hygienic quality of farmhouse meat pies produced in Burkina Faso. Adv Environ Biol. 2012,1:2627 – 36 .
3. Octavia S, Lan R. The family Enterobacteriaceae. The Prokaryotes. 2014, 9:223– 86.
4. Kibret M, Tadesse M. The bacteriological safety and antimicrobial susceptibility of bacteria isolated from street-vended white lupin (Lupinus albus) in Bahir Dar, Ethiopia. Ethiopian J Health Sci. 2013,12:19– 26.
5. Garedew L, Hagos Z, Addis Z, Tesfaye R, Zegeye B. Prevalence and antimicrobial susceptibility patterns of Salmonella isolates in association with hygienic status from butcher shops in Gondar town, Ethiopia. Antimicrob Resist Infect Cont. 2015,4:21– 25.
6. Liu G, Bogaj K, Bortolaia V, Olsen JE, Thomsen LE. Antibiotic-induced, increased conjugative transfer is common to diverse naturally occurring ESBL plasmids in *Escherichia coli*. Front Microbiol. 2019,10:2119 – 23
7. McAfee AJ, McSorley EM, Cuskelly GJ, Moss BW, Wallace JM, Bonham MP et al. AM. Red meat consumption: An overview of the risks and benefits. Meat science. 2010, 84:1– 3.
8. Eurosurveillance Editorial Team. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2011 has been published. Eurosurveillance. 2013,18:20449– 52.
9. Hasman H, Agersø Y, Hendriksen R, Cavaco LM, Food DTU, Guerra-roman B. Laboratory protocol for Isolation of ESBL- , AmpC- and carbapenemases - producing *coli*from fresh meat. 2015,3:3–10.
10. CLSI (2018). *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Proposed Guideline*, 3rd. Edn. Wayne, PA: CLSI.
11. Kado CA, Liu S. Rapid procedure for detection and isolation of large and small plasmids. J Bacteriol. 1981,145:1365– 1373.
12. Uzeh RE, Agunlanna SC. *Escherichia coli* O157:H7 contamination of cattle carcass at slaughter from abattoirs in Lagos, Nigeria. Nig J Microbiol. 2019,33: 4803– 10
13. Gwida M, Hotzel H, Geue L, Tomaso H. Occurrence of Enterobacteriaceae in raw meat and in human samples from Egyptian retail sellers. Internat Schol Res Notices 2014, 2014:1– 6
14. Kilonzo-Nthenge A, Rotich E, Nahashon SN. Evaluation of drug-resistant Enterobacteriaceae in retail poultry and beef. Poult Sci 2013, 92:1098–07.
15. Domagala JM. Structure-activity and structure-side-effect relationships for the quinolone antibacterials. J Antimicrob Chemother. 1994, 33:685–706.
16. Yang B, Qiao L, Zhang X, Cui Y, Xia X, Cui S et al. Serotyping, antimicrobial susceptibility, pulse field gel electrophoresis analysis of Salmonella isolates from retail foods in Henan Province, China. Food Control. 2013, 32:228– 35.
17. Bush K, Jacoby GA. Updated functional classification of β-lactamases. Antimicrob Agent Chemother. 2010,54:969– 76.
18. Odumosu BT, Adeniyi BA, Chandra R. Analysis of integrons and associated gene cassettes in clinical isolates of multidrug resistant Pseudomonas aeruginosa from Southwest Nigeria. Annals Clin MicrobiolAntimicrob. 2013,12:29– 32.
19. Carattoli A. Plasmids and the spread of resistance. Intern J Med Microbiol. 2013,303:298– 304.
20. McMillan EA, Gupta SK, Williams LE, Jové T, Hiott LM, Woodley TA et al. Antimicrobial resistance genes, cassettes, and plasmids present in *Salmonella enterica* associated with United States food animals. FrontMicrobiol. 2019,10:832–.39
21. Brolund A. Overview of ESBL-producing Enterobacteriaceae from a Nordic perspective. Infect Eecol Epidemio. 2014, 4: 1– 9.
22. Perez F, Bonomo RA. Can we really use beta-lactam/beta-lactam inhibitor combinations for the treatment of infections caused by extended-spectrum beta-lactamase-producing bacteria? Clin Infect Dis. 2012,54:175–177