Multidrug Resistant *Escherichia coli* O157 Contamination of Beef and Chicken in Municipal Abattoirs of Southwest Nigeria

Isaac Olufemi Olatoye[1, 2], Elizabeth Adesola Amosun [3], Gabriel Adetunji Ogundipe [1]

[1.] Department of Public Health and Preventive Medicine, University of Ibadan, Nigeria
[2.] Paul Allen School for Global Animal Health, Bustard Hall, Washington State University, Pullman WA
[3.] Department of Veterinary Microbiology and Parasitology, University of Ibadan, Ibadan. Nigeria
olatoye@vetmed.wsu.edu; olaotoyevet@yahoo.com

Abstract: Indiscriminate antibiotics usage in food animals and unhygienic meat processing could predispose meat consumers to risks of antibiotic resistant bacterial contamination and infection. This study investigated the contamination of meat from cattle and chicken slaughtered for human consumption with *E. coli* 0157:H7 at the metropolitan abattoirs and slaughtered slabs of selected poultry farms in Lagos and Ibadan, Nigeria. The aim was to compare the prevalence and antibiotic susceptibility patterns across the different locations and climatic seasons. The organism was isolated by cultural method using selective media and confirmed serologically using latex agglutination kits (Oxoid^{R} UK). Antibiotic susceptibility to ten antimicrobial agents was performed by disc diffusion method using commercial Gram negative discs. Out of 800 meat samples collected, the overall prevalence of 17.1% (comprising of 19.8% and 14.5% of beef and chicken respectively) was obtained. The prevalence of *E. coli* O157:H7 in beef from Ibadan and Lagos were 28.5% and 11.0%, while those of chicken from Ibadan and Lagos markets were 13.0% and 14.0%, and from Ibadan and Lagos farms were 18.0% and 13.0% respectively. The prevalence of *E. coli* O157 was significantly higher in beef compared to chicken ($p<0.05$), while during wet season, contamination of beef was also higher than in dry and significantly higher in beef from Ibadan than Lagos abattoir. All the isolates were resistant to one or multiple antibiotics, but the highest resistance of 91.1% was to tetracycline and nine different resistance patterns were observed among the isolates. Indiscriminate antibiotics usage in livestock predisposes meat consumers to risks of antibiotic resistant *Escherichia coli* O157:H7 in southwest Nigeria. Regulatory control of antibiotics usage in livestock production, meat hygiene and pharmaco-epidemiological surveillance in food animals is hereby recommended to ensure consumer safety.

[Olatoye IO, Amosun EA, Ogundipe GAT. **Multidrug Resistant *Escherichia coli* O157 Contamination of Beef and Chicken in Municipal Abattoirs of Southwest Nigeria**. *Nat Sci* 2012;10(8):125-132]. (ISSN: 1545-0740). http://www.sciencepub.net/nature.

Keywords: Escherichia coli 0157: H7; antibiotics; beef; chicken; abattoir; Nigeria.

1. Introduction

There are growing concern of bacterial adaptation and evolution resulting in the emergence of resistant of zoonotic microorganisms in the food and water. Food-borne disease is a global public health concern. Scallan et al., (2011) reported an estimated food borne 48 million illnesses with about 325,000 hospitalizations and 5,000 deaths annually in United States and in the United Kingdom while Gormley et al., (2011) reported about 2429 foodborne outbreaks in England from 1992 to 2008 mostly caused by bacterial pathogens of which the Vero cytotoxin-producing *Escherichia coli* O157 has increasing contribution.

Escherichia coli is a widespread intestinal commensal organism found in human and animal resulting from faecal contamination or contamination during food animal slaughter it is often found in soil, water and foods. However, Shiga toxin-producing *E. coli* (STEC) O157 emerged as a public health threat following its initial identification as a pathogen in a 1982 outbreak of illness associated with the consumption of undercooked ground beef causing hemorrhagic colitis and hemolytic-uremic syndrome (HUS) in humans (Riley et al., 1983). Cattle and other ruminants were identified as the main reservoir for human infections (Nataro and Kaper, 1998). The enteric habitat of *E. coli* in animals provides easy access to animal-derived meats at slaughter and at points downstream in the food production process. In terms of human enteric infections, enterohemorrhagic *E. coli* (EHEC) O157:H7 produces the most severe disease syndrome. Food borne pathogens may possesse the specialized virulence factors (VF) which cause most extra-intestinal infections.

Resistance to other antibiotics was detected as early as new agents were introduced for therapeutic and growth-promotant purposes (Anderson, 1968; Matthew et al., 1998). Antibiotics resistant *E. coli* has been reported for over 50 years (Adesiyun and Kaminjolo, 1992; Lambie et al., 2000). Studies in the UK found that, in the late 1950s, tetracycline resistance was already detectable in *E. coli* isolates from chickens and pigs fed rations

containing less than 100 g tetracycline/ton (Dunlop et al., 1998a; Orden et al., 1999). Antimicrobial resistant foodborne pathogens are acquired primarily through consumption of contaminated food of animal origin or water (Mead et al., 1999). Food chain, especially meat, is a major source of transmission of antimicrobial-resistant organisms to humans causing both intestinal and extra-intestinal disease (Johnson et al., 2003).

The magnitude of the public health burden due to resistant foodborne pathogens is complex and is influenced by a number of variables such as antimicrobial use practices in farming, process control at slaughter, storage and distribution systems, the availability of clean water, and proper cooking and home hygiene, among others (WHO, 2000). The major concern on the public health threat of foodborne illness is infection by antimicrobial-resistant strains that lead to more intractable and severe disease (Helms et al., 2002; Martin et al., 2004). This situation is further complicated by the potential of resistant bacteria to transfer their resistance determinants to resident constituents of the human microflora and other pathogenic bacteria.

Several published data on resistance in *E. coli* originating from foods were reported from isolates cultured from retail raw meat products (Meng et al., 1988; Zhao et al., 2001; Umolu et al., 2006). Available data from USDA-FSIS indicated that 13 million kg of ground beef and 9.5 million kg of beef trimmings were contaminated with *E. coli* O157:H7 in USA between 1999 and 2002 (Sofos, 2008). Resistance to antibiotics is highly prevalent in bacterial isolates worldwide, particularly in developing countries including Nigeria (Okeke et al., 2005; Aibinu et al., 2007). Unhygienic butchering and floor dressing of carcasses for meat is a common practice in Nigeria resulting in carcass contamination with pathogenic microorganisms that could cause zoonotic food poisoning (Umolu et al., 2006, Ojo et al., 2009; Olatoye, 2010). Seasonal variation could affect the degree of contamination of meat from carcases dressed on the floor since waste water run-off or flooding plays an important role in contamination of food and ground water (Gay and Hunsaker, 1993).

White et al., (2004) suggested the need for continuous research on the ecology and epidemiology of major foodborne pathogens, and surveillance of retail food (including meat) products in order to characterize and mitigate food-borne bacterial resistance Developed countries have national surveillance programmes for monitoring of bacterial susceptibility to antimicrobials among zoonotic and commensal bacteria isolated from humans and animals. However, there are no national surveillance programmes on the susceptibility of such bacteria from animals and products in Nigeria. Additionally, other authors reported the need for continuous exploration of risk assessment of the use of antimicrobials in the animal husbandry with regards to the potential public health consequences. (Hald et al., 2004; Phillips et al., 2004). We therefore hypothesised that the practice of indiscriminate use of antibiotics in livestock production results in shedding of resistant foodborne bacteria and the hygiene levels of meat processing thus the prevalence of contamination of the meat destined for public consumption. This study was aimed at isolation and assessment the resistance patterns of meat-borne *E. coli* O15:H7 in beef and chickens processed for human consumption from two cities abattoirs and poultry slaughter points at retail markets and farms in south western Nigeria. the prevalence in beef were also compared during the dry and wet seasons.

2. Material and Methods

2.1 Samples and Sampling Procedures:

A total of 400 replicate meat samples each twenty five grams were randomly obtained by carcass scrapings according to sampling method adopted by Adams et al., (1980). Meat samples, 200 cattle carcasses each from Bodija (Ibadan) and Oko-Oba (Lagos) abattoirs and 200 broiler chickens each from ten poultry farms, Oko-Oba (Lagos) and Mokola (Ibadan) chicken slaughter markets were aseptically collected into sterile sample bags (Whirl-Pak® Nasco, USA) and placed into icebox which was subsequently transported to the Food and Meat Hygiene Laboratory of the Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, for the bacteriology within 24hours. The samples were collected from April 2008 to June 2009.

2.2 Bacterial Isolation

Twenty five grams of the each sample was thoroughly homogenised with 25ml peptone water, a portion was incubated overnight at 37°C. After incubation the broth were examined for cloudiness indicating bacterial growth. A wire loop full of the broth culture was separately inoculated onto 7% sheep blood agar (Oxoid®) and MacConkey agar (Oxoid®). The plates were incubated aerobically at 37°C for 24hrs. The bacterial isolates were identified by their cultural characteristics, morphology, Gram and biochemical reactions according to standard methods described by Barrow and Felthman (1993). Isolates with red to pink colonies (lactose fermenters) yielding Gram negative rods were characterized and further subcultured onto Sorbitol-MacConkey agar plates, and incubated at 37 °C at 18 hours. Colonies that were colourless to pale, flat and smooth, circular

or serrated at the edge were selected as presumptive non-sorbitol fermenting *E. coli* for serological test. The species identification of bacterial isolates was carried out using standard diagnostic (biochemical and serological) procedures described by Barrow and Felthman (1993) and Farmer, (1999).

Table 1. Distribution of *E. coli* 0157 Strains Isolated from Slaughtered Cattle and Chicken Meat

arcass type	Sample location	No of NSF* *E. coli* isolated	No of *E. coli* 0157 isolated (%)
Cattle (beef)	Bodija (dry s.)	45 (n=100)	26 (26.0)
	Bodija (wet s.)	63 (n=100)	31 (31.0)
	Oko-Oba(dry s.)	36 (n=100)	10 (10.0)
	Oko-Oba(wet s)	42 (n=100)	12 (12.0)
Subtotal		186 (46.5%)	79 (19.8)
Chicken	Ibadan (market)	38 (n=100)	13 (13.0)
	Ibadan (farms)	33 (n=100)	18 (18.0)
	Lagos (market)	36 (n=100)	14 (14.0)
	Lagos (farms)	22 (n=100)	13 (13.0)
Subtotal		129 (32.3%)	58 (14.5)
Total		215 (26.9%)	137 (17.1)

2.3 Determination of Antibiotic Susceptibility and resistance pattern of *E. coli* O157:H7 isolates

The *E. coli* O157 isolates were further tested for their susceptibility to antimicrobial agents using the agar disc-diffusion method. The standard disk diffusion method according to Clinical Laboratory Standard Institute, CLSI (2008) guidelines was applied for antimicrobial susceptibility testing. Commercially available Gram negative multo-disks Abtek® comprising of nitrofurantoin (200µg), cefuroxime (25µg), norfloxacin (30µg), cotrimoxazole (25µg), gentamicin (10µg), tetracycline (30 µg), ciprofloxacin (25µg), nalidixic acid (30 µg), chloramphenicol (30 µg) and ampicillin (25 µg) were tested.

E. coli NCTC 10418 was used as control. A loop full of overnight nutrient broth culture of the pure colonies was inoculated onto Mueller Hinton Agar plates, after which antibiotic discs were carefully and aseptically placed on the surface of the agar. The plates were incubated at 37^{O}C for 16-24 hours. Inhibition zone sizes were interpreted using standard recommendations of the Clinical Laboratory Standard Institute (CLSI, 2008).

Statistical Analysis

Results positive for *E. coli* O157:H7 were express in percentages as prevalence rates for both beef and chicken meat. Statistical comparison of the prevalence rates of the different tissues and sources were done by Chi-square methods.

3. Results

Based on colonial morphology, microscopy and the biochemical tests *E. coli* were *isolated* from which *E. coli* O157:H7 serotype was confirmed with the agglutination test. Out of a total of 400 each of beef and chicken samples examined in this study 186 (46.5%) of beef and 129 (32.3%) of chicken samples were identified as *E. coli*. Seventy nine (19.8%) and 58 (14.5%) of isolates from beef and chicken respectively were confirmed positive for *E. coli* O157:H7 serotype. The prevalence of this pathogen in the beef from Bodija metropolitan abattoir were 26% and 31% during dry and wet season respectively while beef from Oko-Oba yielded the prevalence of 10% and 12% during the dry and wet season respectively (Table 1).

Results of the chicken samples showed prevalence of 13% in Ibadan chicken slaughter markets, 18% in broiler from Ibadan farms, 14% in Lagos chicken slaughter market and 13% in chicken from Lagos farms. Beef from Bodija abattoir (Ibadan) had the highest contamination with this organism. Contamination was also highest in the meat obtained during the wet season than the dry season (Figure 1).

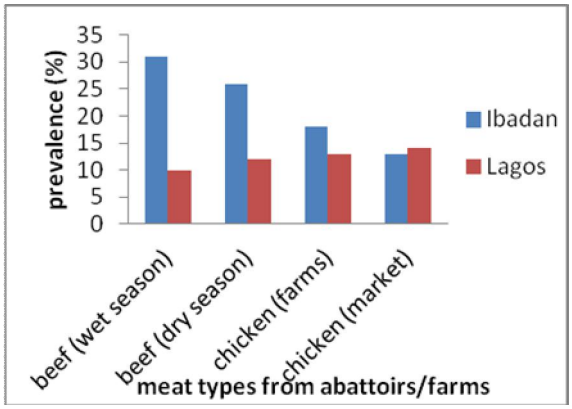

Figure 1. Prevalence of *E. coli* O157:H7 in meat from southwest Nigeria

The prevalence of *E. coli* O157 was significantly higher in beef ($p<0.05$) compared to chicken (19.8% vs. 14.5%). The prevalence of beef at the Bodija abattoir is also significantly higher ($p<0.05$) than that of Oko-Oba abattoir.

Antibiotics Susceptibility and Resistance Pattern of *E. coli* O157 Isolates

Antibiotic susceptibility profile of the The *E. coli* 0157:H7 from beef and chicken characterized in this study displayed resistance to one or more antibiotics as shown in Tables 2 and 3. The resistances rate to tetracycline was the highest in 91.1% and 89.7% of beef and chicken isolates respectively, while norfloxacin, and nalidixic acid had the highest sensitivity (92.4%). These isolates also exhibited variety of multi-resistance (up to nine)

patterns to different combinations of the antibiotics tested as shown in Tables 4 and 5.

Table 2. Frequencies of Antibiotics Resistance of *E. coli* O157:H7 from Southwest Nigeria Abattoirs Beef

Antibiotics	**No of resistant isolates (%)**	**Source of resistant isolates**			
		Bodija (dry season)	**Bodija (wet season)**	**Oko-Oba (dry season)**	**Oko-Oba (wet season)**
Ampicillin	18 (22.8)	8	4	3	3
Cefuroxime	40 (50.6)	12	18	4	6
Ciprofloxacin	6 (7.6)	1	1	2	1
Chloramphenicol	20 (25.3)	7	9	2	2
Cotrimoxazole	15 (19.0)	7	5	2	2
Gentamycin	12 (15.2)	5	4	1	2
Nalidixic	8 (7.6)	3	2	1	2
Nitrofurantoin	60 (80.0)	18	23	8	11
Norfloxacin	15 (19.0)	18	25	9	8
Tetracycline	72 (91.1)	24	29	9	10

Table 3. Frquencies of Antibiotics Resistance of *E. coli* O157:H7 from Southwest Nigeria Abattoirs Chicken Meat

Antibiotics	**No of resistant isolates (%)**	**Source of isolates**			
		Ibadan market	**Ibadan farm**	**Lagos market**	**Lagos farm**
Ampicillin	16 (27.6)	5	3	3	5
Cefuroxime	35 (60.3)	8	8	9	10
Ciprofloxacin	10 (17.2)	2	4	2	2
Chloramphenicol	30 (51.7)	7	11	6	6
Cotrimoxazole	16 (27.6)	5	4	3	4
Gentamycin	15 (25.9)	2	6	3	4
Nalidixic	8 (13.8)	2	1	2	3
Nitrofurantoin	45 (80.8)	8	14	10	13
Norfloxacin	17 (29.3)	3	6	3	5
Tetracycline	52 (89.7)	12	17	12	11

These isolates also exhibited variety of multi-resistance (up to nine) patterns to different combinations of the antibiotics tested as shown in Tables 4 and 5.

Table 4. Antibiotic Resistance Patterns of *E. coli* O157 Isolates from Beef from Lagos and Ibadan Abattoirs during Raining and Dry Seasons

	Pattern of resistance	Sources of the isolates			
		Bodija (dry s.)	Bodija (wet s.)	Oko-Oba (dry s.)	Oko-Oba (wet)
1.	Am,C,Cf,Co,Gn Na,Nf,Te	4	6	2	0
2.	Am,C,Cf,Co,Gn,N,Te	3	5	0	3
3.	Am,C,Cf,N,Na,Te	5	3	0	3
4.	Am,C,Cf,Cp,N,Te	3	2	2	0
5.	Am,C,Co,N,Te	0	5	0	2
6.	Am,C,N,Te	2	3	1	1
7.	Am,C,Cf,N	1	0	3	0
8.	N,Cp,Nf,Te	2	4	0	2
9.	Te	6	3	2	1
	Total	26	31	10	12

Table 5. Antibiotic resistance patterns of *E. coli* isolated from chicken from Lagos and Ibadan abattoirs and farms

	Pattern of resistance	Sources of chicken isolates			
		Ibadan chicken (market)	Ibadan chicken (farms)	Lagos chicken (market)	Lagos chicken (farms)
1.	Am,C,Cf,Co,Gn Na,Nf,Te	4	3	1	3
2.	Am,C,Cf,Co,Gn,N,Te	0	1	1	2
3.	Am,C,Cf,N,Na,Te	2	0	2	0
4.	C,Cf,Cp,N,Te	0	2	0	2
5.	Am,C,Co, Gn,N,Te	3	3	3	4
6.	Am,C,N,Te	0	2	6	2
7.	Am,C,Cf,N	2	3	2	0
8.	N,Cp,Nf,Te	1	0	0	1
9.	Te	1	4	2	1
	Total	13	18	14	15

4. Discussions

During the past two decades, severe outbreaks of gastrointestinal illness have occurred due to food borne pathogenic *E. coli*, especially 0157:H7 (Armstrong et al. 1996). Cattle are reported as the primary reservoir of E. coli 0157:H7 however, the organism has also been isolated from domestic animals such as chicken, pig, sheep and goat (Aibinu et al., 2007; Ojo et al., 2009). In South western Nigeria, Ojo et al., (2009) isolated *E. coli* 0157:H7 strains in the faeces of sheep, goats, pigs and cattle sampled from farms, markets and abattoirs. Aibinu *et al*, 2007 also isolated *E. coli* O157 from cattle, pig, chicken sheep and humans in Lagos and Ogun State in Nigeria. In this present study we isolated *E. coli* 0157 strains in 14.5 % of the chicken meat samples and 19.8% of beef samples.

These results confirmed the high rates of contamination of meat processed at the different meat production centres with *E. coli* which is an indication of the presence of unacceptable levels of other pathogenic microorganisms. The high level of carcass contamination could have resulted from unhygienic slaughtering and meat processing engaged at these abattoirs and slabs, where butchering of meat are mostly done on concrete floor with inadequate slaughtering basic facilities including lack of potable water (Umolu et al., 2006). More so, the prevalence was highest during the wet season could have resulted from environmental contamination by run-off water and flood were transferred to the meat during the butchering and meat dressing. The poor hygiene and lack of the practice of hazard analysis critical control programme (HACCP) at the slaughtering and butchering processes favour such meat contamination (Turtura et al., 1990). This study therefore confirmed that hygiene levels at different abattoirs, with higher prevalence of from Bodija abattoir at Ibadan than Oko-Oba abattoir in Lagos. Oko-Oba abattoir is better organised with modern facilities than other abattoir in Nigeria including Bodija abattoir (Ibironke, 2010).

This study also indicated cattle as a major reservoir of EHEC and antimicrobial-resistant organisms for meat consumers which have also been isolated from live cattle, meat and milk from other parts of the country by different researchers (Ojo et al., 2009; Aibinu et al., 2007; Luga et al., 2007). Cross contamination of ready-to-eat foods and food handlers with such organisms as well as other pathogenic bacteria could result from poor personal hygiene (Muhammad et al., 2009) and the habit of eating at the abattoirs during meat processing by the butchers.

All the *E. coli* O157 isolated in present study exhibited multiple resistances to all the antibiotics used in the study. Similar findings on multiple drug resistance of *E. coli* strains have been reported from Nigeria and other parts of the world (Ojo et al., 2009; Rahman et al., 2008; Umolu et al., 2006; Aibinu et al., 2007; Zhao et al., 2005; Khan et al., 2002). The highest resistance prevalence to tetracycline obtained in this study also agrees with the report of Aibinu et al., 2007 who obtained 94.4% resistance prevalence to tetracycline in isolates from animals and man. Umolu et al., 2006 isolated multiple resistant strains of *E. coli* in meat from slaughtered cattle in Edo State, Nigeria. Daini and Adesemowo, (2008) also found the resistance of *E. coli* from Nigeria in 54% and 88% strains against gentamicin and tetracycline respectively. Also high resistance of enterotoxigenic *E. coli* has been reported by other authors across the globe, for example in England and Wales about 30% isolated *E. coli* from pigs were multidrug resistant while in Canada, 93% *E. coli* isolates were resistant to tetracycline, and a similar number (91%) were resistant to sulphonamides(Matthew et al., 1998). The high prevalence of antibiotic resistant bacteria in Nigeria and other developing countries has been associated with several factors including

indiscriminate use due to unregulated access of non-professional to different classes of antimicrobial over-the-counter (Okeke et al., 1999; Kabir et al., 2004). Tetracycline which has the highest resistance in this study is one of the most commonly available for use as growth promoter and routine chemoprophylaxis among livestock in Nigeria. They are readily available in different dosage forms and in combination with other antibiotics and vitamins. Antimicrobial use and misuse have been considered to be the most vital selecting force to antimicrobial resistance of bacteria development and spread in both veterinary and human medicine (Witte, 1998; Okeke et al., 2005).

The public health significance of these findings is that antimicrobial resistant bacteria from food animals may colonize the human population via the food chain, contact through occupational exposure, or waste runoff from meat production facilities to the neighborhood

There is increasing evidence that treatment of STEC infections by antibiotics should be discouraged because they may increase Shiga toxin release. The results of antibiotic resistance in EHEC contaminating meat obtained in this study support the rationale for discouraging antibiotic treatment of EHEC infections for food safety purpose.

Conclusion

This study showed that high proportion of beef and chicken sold for human consumption in the studied area were contaminated with antibiotic resistant *E. coli* O157:H7 with variable prevalence in both chicken and beef across the abattoirs and during the different climatic seasons. The results showed zoonotic microbial hazards of unhygienic meat processing commonly practiced in Nigeria abattoirs and the contributions to global epidemiology of bacterial resistance.

The public health and food safety implications of these results include the risk of meat borne food poisoning and spread of antibiotic resistance across the food chain and meat processing plants. Hygienic meat production and processing practices should be promoted among the butchers and other meat handlers. The improvement of abattoir facilities such as slaughter and butchering line, as well as provision of potable water and laboratory infrastructures and the practice of HACCP with pharmaco-epidemiological surveillance are also recommended. Regular water treatment such as chlorination will help to reduce the incidence *E. coli* in livestock farms and abattoir water.

Acknowledgements:

Authors are grateful to (1) MacArthur Foundation for oversea travel grant support and SGAH, Washington State University, USA for training opportunity and materials provided for this work.

Corresponding Author:
Dr. Isaac Olufemi Olatoye
Department of Veterinary Public Health & Preventive Medicine, University of Ibadan, Nigeria.
Paul Allen School for Global Animal Health, Washington State University, Pullman, WA USA
olatoye@vetmed.wsu.edu; olaotoyevet@yahoo.com

References

1. Adesiyun AA, Kaminjolo JS. Susceptibility to antibiotics of *Escherichia coli* strains isolated from diarrhoeic and non-diarrhoeic livestock in Trinidad. Revue Elev Med Vet Pays Trop. 1992:45:260-262.
2. Aibinu IE, Peters RF, Amisu KO, Adesida SA, et al. Multidrug Resistance in *E. coli* O157 Strains and Public Health Implication. J Animal Science. 2007:3(3):22-33.
3. Anderson ES Drug Resistance in Salmonella typhimurium and its Implications. *Brit Med J* 1968; 3:333-339
4. Armstrong GL, Hollingsworth J, Morris JG. Emerging foodborne pathogens: *Escherichia coli* 0157:H7 as a model of entry of a new pathogen into the food supply of the developed world. Epidemiology Review. 1996:18:29-51.
5. Barrow GT and Feltham RKA. Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd ed. Cambridge University Press, Cambridge 1993:
6. CLSI. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals: Approved standard CLSI document M31-A3, 3rd edition. Clinical and Laboratory Standard Institute, Wayne, PA 2008.
7. Daini OA and Adesemowo A. Antimicrobial susceptibility pattern and r- plasmids of clinical strains of *Escherichia coli*. Australia Journal of Basic and Applied Science 2008:2(3):397-400.
8. Dina OA, Arowolo ROA. Some Considerations on veterinary drug use and supply in Nigeria. Re. d'Elevage Med. Vet. Pays Tropicaux 1991:44:29-31.
9. Dunlop RH, McEwen SA, Meek AH, Clarke RC, Black WD, Friendship RM. Associations among antimicrobial drug treatments and antimicrobial resistance of fecal *Escherichia coli* of swine on 34 farrow-to-finish farms in Ontario, Canada.

Preventive Veterinary Medicine 1998:34(4):283-305.
10. Gay JM, Hunsaker ME. Isolation of multiple *Salmonella* serovars from a dairy two years after a clinical salmonellosis outbreak. Journal America Veterinary Medical Association 1993:203:1314–1320.
11. Gormley FJ, Little CL, Rawal N, Gillespie IA, Lebaigue S, Adak GK: A 17-year review of foodborne outbreaks: describing the continuing decline in England and Wales (1992–2008). Epidemiology of Infections 2011:139:688-699.
12. Hald B, Skovgaard H, Bang DD. Flies and *Campylobacter* infection of broiler flocks. Emerging Infectious Diseases 2004:10:1490-1492.
13. Hart CA, Kariuki S. Antimicrobial resistance in developing countries British Medical Journal 1998:317:647-650.
14. Helms M, Vastrup P, Gerner-Smidt P, Molbak K. Excess mortality associated with antimicrobial drug resisitant *S. typhimurium*. Emerging Infectious Diseases 2002:8(15):490-495.
15. Ibironke AA. A retrospective study of liver fluke disease in cattle based on abattoir data in Agege, Lagos, Nigeria. Africa Journal Food Agriculture and Natural Development 2010:10:10:1-5.
16. Johnson JR, Murray AC, Gajewski A. Isolation and molecular characterization of nalidixic acid-resistant extraintestinal pathogenic *Escherichia coli* from retail chicken products. Antimicrobial Agents Chemotheraphy 2003:47:2161-2168.
17. Khan A, Das SC, Ramamurthy T, Sikandar A. Antibiotic Resistance, Virulence Gene, and Molecular Profiles of Shiga Toxin-Producing *Escherichia coli* Isolates from Diverse Sourecs in Calcutta, India Journal of Clinical Microbiology 2002:40(6):2009-2015
18. Lambie N, Ngeleka M, Brown G, Ryan J. Retrospective study on *Escherichia coli* infection in broilers subjected to postmortem examination and antibiotic resistance of isolates in Trinidad. Avian Disease 2000:44:155-60.
19. Levine M. *Escherichia coli* that cause diarrhea; enterotoxigenic, enteropathogenic, enteroinnvasive, enterohemorrhagic and enteroherent. Journal of infectious Disease 1987:155:377-390.
20. Luga II, Akodu I, Mhomga I, Allam L, Ajogi I, Umoh VJ, Kwaga JKP. Antimicrobial resistance of shigatoxin producing *Escherichia coli*, O157: H7 O157 NM isolates from water fed to cattle in northwestern, Nigeria. Asian Journal Animal and Veterinary Ad 2007:2:205-211.
21. Martin LJ, Fyfe M, Dore K, Buxton JA, Pollari F, Henry B, Middleton D, Ahmed R, Jamieson F, Ciebin B, McEwen SA, Wilson JB. Multi-Provincial Salmonella Typhimurium Case-Control Study Steering Committee: Increased burden of illness associated with antimicrobial-resistant *Salmonella enterica* serotype *typhimurium* infections. Journal of Infectious Disease 2004:189:377–384.
22. Matthew AG, Upchurch W, Chattin SE. Incidence of antibiotic resistance of fecal Escherichia coli isolated from commercial swine farms. Journal of Animal Science 1998:76:429-434.
23. Mead P, Slutsker L, Dietz V, McCaign L, Bresee J, Shapiro C. Food-related illness and death in the United States. *Emerging Infectious Disease* 1999:5:607-25.
24. Meng, J. and Doyle, M.P. 1997. Emerging issues in microbiological food safety. Annual Reveiw Nutrition 1999:17: 255–75.
25. Muhammad AA, Saidul Alam SMR, Hassan MS. Antibiotic Resistance of *Escherichia Coli* Isolated From Poultry and Poultry Environment of Bangladesh. Internet Journal of Food Safety 2009:11:19-23
26. Nataro JP, Kaper JB Diarrheagenic *Escherichia coli.* Clinical Microbiology Review 1998:11:142–201.
27. National Research Council Institute of Medicine. Issues specific to antibiotics. In: National Research Council Institute of Medicine. The Use of Drugs in Food Animals: Benefits and Risks. Washington, D.C. National Academy Press; 1998:120-150.
28. Ojo OE, Oyekunle MA, Ogunleye AO, Otesile EB. *E. coli* 0157:H7 in food animals in part of S/Western Nigeria: Prevalence and invitro antimicrobial susceptibility. Tropical Veterinarian 2009:26: 23-30.
29. Okeke IN, Laxminarayan R, Butta ZA, Duse AG, Jenkins P, O'Brien TF, Pabloso-Mendez A, Klugman, KP. Antimicrobial resistance in developing countries. Part i Recent Trends and current status. Lancet Infectious Disease 2005:5:481-493.
30. Okeke IN, Adebayo L and Edellman R. Socioeconomics and Behavioural Factors Leading toAcquired Bacterial Resistance to Antibiotics in Developing Countries. Emerging Infectious Disease 1999:5:18-27.
31. Olatoye IO. The incidence and antibiotics susceptibility of *Escherichia coli* O157:H7 from beef in Ibadan Municipal, Nigeria. African Journal of Biotechnilogy 2010:9(8):1196-1199.
32. Orden, J, Ruiz Santa Quiteria J, Garcia S, De La Fuente R. In vitro activities of *E. coli* strains isolated from diarrhoeic dairy calves.

Antimicrobial Agents and Chemotherapy 1999:43:510-513.
33. Phillips I, Casewell M, Cox T, De Groot B, Friis C, Jones R, Nightingale C, Preston R, Waddell J. Does the use of antibiotics in food animals pose a risk tohuman health? A critical review of published data. Journal of Antimicrobial Chemotheraphy 2004:23:28–52.
34. Rahman D, Khan MK, Malik K. Antibiotic Resistance and Detection of β-lactamase in Bacterial Strains of *Staphylocci* and *Escherichia coli* isolated from foodstuffs. World Journal of Microbiology and Biotechnology 2001:17:863-868.
35. Riley L.W., Remi, R.S., Helgerson S.D., Mc Gee, H.B., Wells, B.K., Davis, R., Hebert, J., Olcott, E.S., Johnson, L., Hargrett, N.T., Blake, P.A., Cohen, M.L. Haemorrhagic colitis associated with a name *E. coli* serotype. New England Medicine 1983:24:681-685.
36. Scallan R, Hoekstra M, Angulo FJ. 2011: Foodborne illness acquired in the United States--major pathogens. Emerging Infectious Disease 2011: 17, 1-15.
37. Sofos JN. Challenges to meat safety in the 21st century. Meat Science 2008:78:3-13.
38. Turtura GC, Massa S, Chazvinizadeh H. Antibiotic resistance among coliform bacteria isolated from carcasses of commercially slaughtered chickens. Int J Food Microbiol 1990:11:351–3544.
39. Umolu PI, Ohenhen ER, Okwu IG, Ogiehor IS. Multiple Antibiotics Resistant Index and Plasmid of Escherichia coli in Beef in Ekpoma. Journal of American Science 2006:2(3):22-28.
40. White DG, Zhao S, Singh R, McDermott PF. Antimicrobial resistance among gram-negative foodborne bacterial pathogens associated with foods of animal origin. Foodborne Pathogens and Diseases 2004 1:137-152.
41. World Health Organization. WHO Global Principles for the Containment of Antimicrobial Resistance in Animals Intended for Food. Report of a WHO Consultation with the participation of the Food and Agriculture Organization of the United Nations and the Office International des Epizooties 2000; WHO/CDS/CSR/APH/2000.4 http://www.who.int/emc accessed on 21st December, 2009.
42. Witte W. Medical Consequences of Antibiotic Use in Agriculture. Science 1998 279:(53):996-997.
43. Zhao S, White DG, Ge B, Ayers S, Friedman S, English L, Wagner D, Gaines S, Meng J. Identification and characterization of integron-mediated antibiotic resistance among Shiga toxin-producing *Escherichia coli* isolates. Appl Environ. Microbiol 2001:67:1558-1564.

7/19/2012