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Prevalence and Plasmid Profile **of** Fluoroquinolone-Resistant **Escherichia coli isolates from Domestic** Animals **in Enugu State, Nigeria**

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Abstract
Plasmid analysis is an important technique for the determination and characterization of antibiotic resistance traits in procaryotes. This study is aimed at investigating the prevalence and plasmid profile of fluoroquinolone resistant *E. coli* isolated from domestic livestock in Enugu State, Nigeria. A total of 559 *Escherichia coli* were isolated from pig, cattle and chicken in Enugu State, Nigeria These isolates were screened for antibiotic susceptibility and plasmid profiles. Thirty four (34) of 559 (6.0%) of the isolates were found to be fluoroquinolone resistant. The prevalence of fluoroquinolone resistance among the *E.coli* isolates from the animals tested were: pig (5.7, 6.1, 5.7 and 7.8%), cattle (0, 0, 0 and 0%) and chicken (13.6, 14.3, 11.6 and 17.7%) for ciprofloxacin, ofloxacin, levofloxacin and pefloxacin respectively. Plasmid of different sizes were detected in the isolates. Out of the 24 plasmids detected in the FQREC animal isolates, 9 different profiles were recorded; 3 in pigs and 6 in chicken. Isolates with high multi-drug resistance profiles were found to possess multiple plasmids with large sizes in the range of 2026 to 23130 bp. The plasmids were cured to the range of 40 - 80% depending on the source of the isolates thus, confirming the contribution of plasmid in mediating fluoroquinolone resistance in animal FQREC isolates. There was high prevalence of fluoroquinolone-resistant animal *E. coli* isolates in the studied districts and may constitute a potential reservoir of resistance plasmids that could be transferred to pathogenic bacteria.
Keywords. Prevalence, fluoroquinolone, Plasmid profile, *Escherichia coli*, Domestic animals, Enugu State, Nigeria.

Introduction

Escherichia coli is a Gram negative rod-shaped bacterium that is commonly found in the intestines of humans and animals. Fluoroquinolone antibiotics have helped and are still currently in use in the treatment and prevention of diseases in domestic animals, however, there is a growing awareness of public health concerns associated with the use of these antibiotics (Van derAuwera *et al.*, 2009). The widespread use of various antibiotics for treating animal infections has created antibiotic resistant bacterial strains (Tivendale *et al*., 2009; Sackey *et al.*, 2001). Antibiotic-resistant *Escherichia coli* has been shown to be less common on poultry raised without antibiotics as compared to poultry raised conventionally (Zhang *et al*., 2011). Likewise, organic poultry can have lower frequencies of antibiotic-resistant bacteria than poultry raised conventionally (Miranda *et al* 2007, Miranda *et al*., 2009). In intensively reared food animals, one of the common practices is that antibiotics are administered to whole flocks rather than individual animals, and antimicrobial agents may be continuously fed to food animals such as poultry, pig and cattle as antimicrobial growth promoters. Therefore, the antibiotic selection pressure for bacterial drug resistance in animal is high and invariably their faecal flora contains a relatively high proportion of resistant bacteria (Whiteworth *et al*., 2008). Carraminana *et al*. (2004) isolated some bacterial strains from a poultry slaughter

house in Spain and recorded high percentages of resistance to many antibiotics such as neomycin (53.4%), tetracycline (21.8%), and streptomycin (11.3%). Nsofor and Iroegbu (2012) reported very high frequency of resistance to ampicillin (85%), cotrimaoxazole (90%), cephalothin (90%) and streptomycin (77.5%) in *Escherichia coli* strains isolated from animals in Nigeria. A significant relationship between fluoroquinolone use and resistance to these antibiotics has been documented (Goettsch *et al*., 2000). This acquired resistance occurs not only in pathogenic bacteria but also in the endogenous flora of exposed individual animals.

Plasmids are major mechanism for the spread of antibiotic resistant genes in bacterial populations (Fang *et al*., 2008). The spread of antibiotic resistance plasmids in *E. coli* from chickens to human handlers or of antibiotics-resistant microorganisms from animal to humans in various countries has been reported (Fang *et al*., 2008). Conjugation occurs by F-plasmids that can transfer genes encoded for multiple resistance and mobilize other non-conjugative plasmids to host cells. Multiple antibiotic resistance genes are harbored on R-plasmids some of which are conjugative (Pitout *et al.*, 2009). *E.coli* have been reported to transfer the antibiotic resistant genes to enteric pathogenic and normal flora bacteria, such as, *Salmonella spp* and *Proteus spp* (Yoon and Hovde, 2008). The objective of this study was to investigate the prevalence and plasmid profile of fluoroquinolone resistant *E.coli* isolated from domestic live stock in Enugu State Nigeria.

Materials and methods

All the animals (cattles, pigs and chickens) included in the study were obtained from various herds, poultry houses and piggeries within Enugu State. These animals, at the time of specimen collection, were not showing any sign of ill-health. There was no documented evidence of antibiotics use in the farms from which the specimens were collected. Non duplicate fresh fecal droppings, nasal swabs, skin swabs and meat/vendor's table swabs were randomly collected from cattle, pigs, and chicken into clean, labelled screw capped tubes and packed in iceboxes. These specimens were transported to the Microbiology Laboratory of the Department of Pharmaceutical Microbiology, University of Nigeria, Nsukka for immediate culture and sensitivity tests. *E.coli* strains were isolated and identified by standard microbiological methods (Cheesbrough, 2000). The identity of the bacteria was confirmed by DNA sequencing analysis on the samples sent to and done at Inqaba Biotechnology Pty South Africa. Antibiograms were prepared for the *E. coli* isolates against ciprofloxacin (5 µg), ofloxacin (5 µg), levofloxacin (5 µg), pefloxacin (5 µg) gentamicin (30 µg), ceftriaxone (30 µg), amoxicillin (25 µg), erythromycin (15 µg) and tetracycline (30 µg) by disk diffusion method according to Clinical Laboratory Standard Institute (CLSI) recommendations (CLSI 2006) on Mueller-Hinton agar plates. All culture media and antibiotics were from Oxoid, Cambridge, UK). Inhibition zone diameters were measured after 24 h of incubation at 37°C and were interpreted using the breakpoints of the Clinical Laboratory Standard Institute (CLSI, 2006). A standard *E. coli* (ATCC 25922) was used as a control.

Plasmid profiling

The test fluoroquinolone resistant isolates were evaluated for the presence of plasmid DNA as described elsewhere (Gohar *et al*., 2015). One ml of 24 h cultures of test organisms in Trypcate Soy Broth (TSB) medium (Merck, Germany) was transferred into 1.5ml sterile Eppendorf micro-fuge tubes and centrifuged at 10.000g for 10min. The resultant pellets were dissolved in 600µl of lysis buffer (Nacl 1M, Tris – HCL 1M, EDTA 0.5M), 20µl SDS (25%), 3 µl of proteinase – K (20mg/ml) and incubated at 60^{o}C for 1 h. After the lysis, 620 µl of phenol/chloroform/isoamylalcohol (25:24:1 volume/volume) was added to the above solutions, vortexed and centrifuged at 12.00g for 10min. The supernatants were aseptically transferred to sterile micro-fuge tubes to which 1ml of 95% cold ethanol was added. The micro-fuge tubes were allowed to stand for 1h in refrigeration condition (4^{o}C). Plasmid DNA were precipitated in each tube by centrifugation at 12.00g for 10 mins. The precipitated DNA was dissolved in 50 µl of 10mM Tris EDTA – buffer (TE) containing 10 µl of RNASE. The plasmids were run on 1.5% agarose gel electrophoresis and visualized under UV light transilluminator and photographed as described by other authors (Farshad et al., 2010).

Plasmid curing experiment:

Plasmid curing was conducted by treating FQREC isolates with sub-inhibitory concentrations : 0.1, 0.2, 0.3, 0.4 and 0.5mg/ml of acridine orange in Mueller-Hinton broth according to a previously described method (Esimone *et al*., 2010). The tubes were incubated at 37^{o}C for 24 h. After incubation, the broth was shaken properly for homogenization of the culture, and loopful of the broth culture taken and inoculated onto drug-free Mueller-Hinton agar plates and incubated for 24 h at 37^{o}C. Susceptibility tests were carried out on the FQREC isolates using some selected antibiotic disks, and then, the diameter of zones of inhibition were taken after incubation.

Result

Prevance of fluoroquinolone resistance among *Escherichia coli isolates*

In this study, a total of 559 *E. coli* isolates from three domestic livestock comprising of pigs, cattles and chickens were tested against nine antibiotics. Thirty four (34) of 559 (6.0%) of the isolates were found to be fluoroquinolone resistant. The prevalence of fluoroquinolone resistance among the *E. coli* isolates from the animals tested were: pig (5.7, 6.1, 5.7 and 7.8%), cattle (0, 0, 0 and 0%) and chicken (13.6, 14.3, 11.6 and 17.7%) for ciprofloxacin, ofloxacin, levofloxacin and pefloxacin respectively (Table i). For non fluoroquinolone antibiotics, the 244 pig, 168 cattle and 147 chicken *E. coli* isolates tested showed respective percentage resistance to gentamicin, ceftriaxone, amoxycillin, erythromycin and doxycycline in: pig (4.9, 2.9, 64.8, 73.0, 63.5%), cattle (3.6, 1.8, 57, 90.5, 64.9%) and chicken (16.3, 10.9, 88.0, 96, 87.1%). All isolates were resistant to at least three or more antibiotics. The prevalences of FQREC isolates are shown in Table ii. No FQREC was isolated from the nasal swabs of the pigs and all the specimens from cattle. The mean prevalences of FQREC from the *E. coli* isolates from the nasal swab, fecal specimen, skin swab and meat/vendor's table swab of pig were 0, 9.2, 10.3 and 11% respectively. For chicken *E. coli* isolates, the mean prevalences of FQREC from the fecal specimen, skin swab and meat/vendor's table swab were 13.2. 14.0 and 16.7% respectively.

Plasmid profile Result

A total of 24 plasmids were detected from the animals FQREC isolates (Table 2). Some isolates harbored one or more plasmids of different molecular weight ranging from 2.0 – 23.1 KB. Of these 24 plasmids detected , 9 different profiles were recorded; 3 in pigs and 6 in chickens. The 23.1 KB plasmid was most frequent in the animals occurring at the rate of 57.1% in pig and 52.9% in chicken. The 504 bp plasmid was absent in both animals. The 2322 , 4361 and 9216 bp were present in FQREC isolates from chicken specimens but, not detected in pigs. Figures 1 and 2 below show gel images of some of the plamid profiles of FQREC isolates from pigs and chickens respectively.

Table i. The antibiotics resistance profile of *E.coli* isolates from the animals tested.

	Number of *E. coli* isolates (%) prevalence									
Animals	**Number of isolate(y)**	**CPX**	**Ofx**	**Lev**	**Pef**	**Gn**	**Cef**	**Amx**	**Ery**	**Doxy**
Pig	244	14	15	14	19	12	7	158	178	155
	(14.5)	(5.7)	(6.1)	(5.7)	(7.8)	(4.9)	(2.9)	(64.8)	(73)	(63.5)
Cattle	168	0	0	0	0	6	3	96	152	109
	(10.0)	(0)	(0)	(0)	(0)	(3.6)	(1.8)	(57)	(90.5)	(64.9)
Chicken	147	20	21	17	26	24	16	130	141	128
	(8.8)	(13.6)	(14.3)	(11.6)	(17.7)	(16.3)	(10.9)	(88)	(96)	(87.1)

Table ii : Prevalence (%) of fluoroquinolone –resistant *E. coli* (FQREC) isolates from the test animals according to the specimen source.

Animal.	Source .	No of *E .coli* isolate (y%)	Prevalence of FQREC (%) Cpx	ofx	lev	pef
	Nasal swab	38(10.8)	0 (0)	0(0)	0(0)	0 (0)
Pig	Fecal specimen	87(24.9)	7 (8.1)	8 (9.2)	7 (8.1)	10(11.5)
	Skin swab	39(11.1)	4(10.3)	4(10.3)	4(10.3)	4(10.3)
	Meat/vendors table	32 (9.1)	3(9.4)	3(9.4)	3(9.4)	3(15.6)
	Nasal swab	25(7.1)	0 (0)	0 (0)	0 (0)	0 (0)
Cattle	Fecal specimen	68(19.4)	0 (0)	0 (0)	0 (0)	0 (0)
	Skin swab	27(7.7)	0 (0)	0 (0)	0 (0)	0 (0)
	Meat/vendors table	48(13.7)	0 (0)	0 (0)	0 (0)	0 (0)
Chicken	Fecal specimen	83(23.7)	10(12.0)	11(13.2)	10(12.0)	13(15.7)
	Skin swab	25(7.1)	3(12.0)	3(12.0)	3(12.0)	5(20.0)
	Meat/vendors table	38(11.1)	7(17.9)	7(17.9)	4(10.3)	8(20.5)

NB; values in parenthesis under the number of isolates represent prevalence of *Escherichia coli* (y) and FQREC
Key: CPX= Ciprofloxacin, Lev= Levofloxacin , OFX = Ofloxacin, Pef = Pefloxacin

Table iii. The Distribution of Plasmids in FQREC according to specimen source in animals

Plasmid Size (bp)	Frequency of Distribution according to specimen Source AHP 7(%)	AHC 17(%)
504	0(0.0)	0(0)
2027	1(14.3)	2(11.8)
2322	0(0)	1(5.9)
4361	0(0)	2(11.8)
6557	2(28.6)	1(5.9)
9216	0(0)	2(5.9)
23130	4(57.1)	9(52.9)

Key:
AHP = apparently healthy pig
AHC = apparently healthy chicken

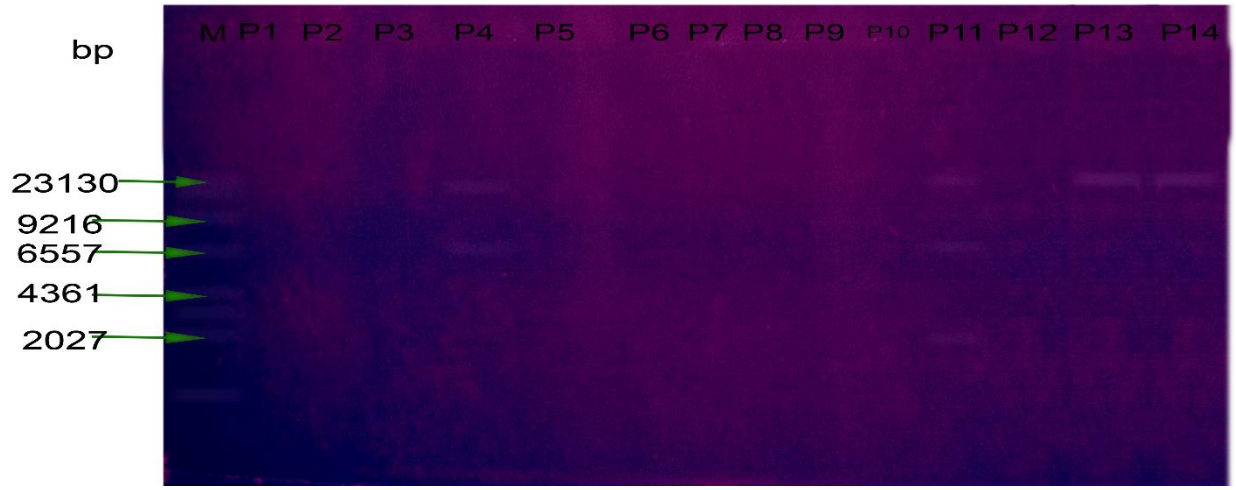

Figure 1: The Image of gel electrophoresis of Plasmid DNA from the FQREC isolates from pig in the study area.
Lane M is HIND III Marker, lanes with and without bands are shown above.

Figure 2: The Image of gel electrophoresis of Plasmid DNA from the FQREC isolates from the second group of chicken in the study area.
Lane M is HIND III Marker, lanes with and without bands are shown above.

Discussion

Domestic animal growers use antibiotics both for therapeutic purposes and for growth promotion (MacDonald and Wang, 2011,). The use of antibiotics in poultry production can select for antibiotic-resistant microorganisms including *Salmonella*, *Campylobacter*, *Enterococcus*, and extra-intestinal pathogenic *E. coli* (Davis 2011).

In our studies, the prevalences of fluoroquinolone resistant *E.coli* isolates from chicken and pig are high and greater than that of cattle isolates. One of the causes of this is that the use of fluoroquinolone is more in chicken than in the pig or cattle in the study area. In addition, human handling of chicken is high and more frequent (as they are more friendly) than with pigs and cattle and in so doing, resistant strain /gene may be transferred from human to animals. Moreso, the cattle mainly feed on grasses and the use of fluoroquinolones in cattle is minimal. The high fluoroquinolone resistant rate found in chicken and pig *E.coli i*solates in this study could be due to the inappropriate use of the drug in treatment and prevention of their diseases as well as their use as poultry growth promoters in the study area. Other researchers in other parts of the world have indicated that fluoroquinolone resistance in *E.coli i*solates is increasing (Kariuki *et al.*,2007; Karlowsky *et al*., 2006). To the best of our knowledge, this is the first report in Enugu State showing the prevalence of fluoroquinolone resistance in chicken isolates being higher than isolates from pigs or cattle. Based on the specimen type, the highest prevalence of FQREC was obtained from specimen of skin swab in pig and meat/vendor's table in chicken and the least prevalence was obtained from the fecal isolates in both animals. Contamination of meat from the meat vendor's table and the meat sellers himself might have contributed to the value of prevalence of isolates from meat/vendor's table being higher than the prevalence of isolates from other specimen sources in chicken. The prevalence of FQREC from pig skin swab isolates was the highest when compared with other specimen types in the pigs tested. The reason for this may not be unconnected with the pig's lifestyle of living in the dirty environment and the fluoroquinolone resistant genes are easily transfered from other environmental bacteria to the *E coli* found on the skin of pigs.

In this study, plasmid profile analysis of the FQREC isolates by agarose gel electrophoretic techniques showed a total of 24 different plasmid bands occurring in various combinations. The size of these bands ranged from 2.0 to 23.1 KB and most of the plasmids were shared among animal isolates. According to a work done by Uma *et al.*, (2009), the plasmid sizes ranged from 1.0 to 25 kb, the most common plasmid of size 4.8 kb being detected in all the plasmid-harbored *E. coli* strains. The

slight variation in results may be due to difference in origin of isolation of *E. coli*, geographical distribution of the bacteria and exposure to different antimicrobials. Our findings revealed that our test isolates with

high multi-drug resistance profiles were found to possess multiple plasmids with large sizes ranging from 2.0 – 23.1 KB. This shows that there exists correlation between plasmid size and number to that of antibiogram of the isolates. In *E.coli* strains, antibiotics resistance increases as a function of time and their exposure to many agents such as chemicals, biocides, antibiotics, etc. In this study, the greater percentage of the plasmids fall between 6.6 KB and 23.1 KB , this is similar to previous reports by Uchechi and Erinma, (2007) in south eastern Nigeria. The plasmids were cured to the range of 40 - 80% depending on the source of the isolates thus, confirming the contribution of plasmid in mediating fluoroquinolone resistance in animal FQREC isolates.

In conclusion, this high prevalent fluoroquinolone resistant animal *E. coli* isolates constitute a potential reservoir of resistance plasmids that could be transferred to pathogenic bacteria. The findings of this study provide evidence to corroborate the existence of a reservoir of antibiotic resistance genes in animals. Infections with fluoroquinolone resistant pathogens limit the options available to treat infectious diseases of animals and humans. The high prevalence of fluoroquinolone resistant *E.coli* observed in this work may act as a 'catalyst' for an urgent need of health education and communication especially as it concerns antibiotic use in veterinary medical practise in Enugu State and Nigeria at large.

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References

Carraminana, J. J., Rota, C., Augustin, I., Herrera, A. (2004). High prevalence of multiple resistance to antibiotics in Salmonella serovars isolated from a poultry slaughterhouse in Spain. Vet.Microbiol. 104: 133-139.Cheesbrough M (2000). District Laboratory Practice in Tropical Countries, Part 2. Cambridge University Press, Cambridge, UK..434.

Clinical Laboratory Standards Institute (CLSI)(2006): Performance standards for Antimicrobial susceptibility testing. National committee for clinical laboratory standards, W ayne pa

Davis, M. F., Price, L. B and Liu, C. M, *et al* (2011): An ecological perspective on US industrial poultry production: the role of anthropogenic ecosystems on the emergence of drug-resistant bacteria from agricultural environments. *Curr Opin Microbiol.* **14**(3): 244–250.

Fang, H., Ferda, A., Göran, H, Dornbusch, K. (2008). Molecular Epidemiology of Extended Spectrum β-Lactamases among Escherichia coliIsolates collected in a Swedish hospital and its associated health care facilities from 2001 to 2006. *J. Clin. Microbiol.*46(2): 707-712.

Farshad, S., Ranjbar, R., Japoni, A., Hosseini, M., Anvarinejad, M and Mohammadzadegan, R. (2012).Microbial Susceptibility, virulence Factors.

Goettsch, W., van Pelt, W., Nageikerke, N., Hendrix, M. G., Buiting, A.G., Petit, P.L, Sabbe, L.J, van Griethuvsen, A.J, Neelinga, A.J. (2000) Increasing resistance to fluoroquinolones in *Escherichia coli* from urinary tract infections in the Netherlands. *J Antimicrob Chemother* 46: 223-228.

Gohar, A., Sheikh, A.A., Anjum, T., Hussain, J., Muhammad,J., Tabbassum ,A., Kanwal, A., and Kanwal, I. (2015) Plasmid Profiling And Curing Of Multidrug Resistant *Escherichia Coli* Recovered From Retail Chicken Meat; *J. Anim. Plant Sci.*, 25 (4) 984.

Kariuki, S., Revathi, G., Corkill, J., Mwituria, J., Mirza, N. and Hart, C.A. (2007) *Escherichia coli* from community-acquired urinary tract infections resistant to fluoroquinolones and extended-spectrum beta-lactamase. *J. Infect Dev Ctries* 1:257-262, doi:103855/jidc.361.

Karlowsky, J.A., Hoban, dj, de Corby, M.R., Laing, N.M. and Zhanel, G.G. (2006) Fluoroquinolone-resistant urinary isolates of *Escherichia coli* from out patients are frequently multidrug-resistant: results from the North American urinary tract infection collaborative alliance-quinolone resistance study. Antimicrob Agents Chemother 50:2251-2254.

MacDonald, J. M., Wang, S.L. (2011;) Foregoing sub-therapeutic antibiotics: the impact on broiler grow-out operations. *Appl Econ Perspect Policy.* **33**(1): 79–98.

Miranda, J. M., Mondragon, A. Vazquez, B. I,(2009). Microbiological quality and antimicrobial resistance of Escherichia coli and Staphylococcus aureus isolated from conventional and organic "Arzua-Ulloa" cheese. *Cyta-J Food.* **7**: 103–110.

Miranda, J. M., Vazquez, B. I., Fente, C.A. (2008) .Antimicrobial resistance in Escherichia coli strains isolated from organic and conventional pork meat: A comparative survey. *Eur Food Res Technol.* **226**(3): 371–375.

Miranda, J. M., Guarddon, M., Mondragon, A,(2007): Antimicrobial resistance in Enterococcus spp. strains isolated from organic chicken, conventional chicken, and turkey meat: A comparative survey. *J Food Prot.* **70**(4): 1021–1024.

Nsofor CA, Iroegbu C U (2012). Antibiotic resistance profile of Escherichia coli isolated from apparently healthy domestic livestock in South-East Nigeria. *J. Cell Anim. Biol*.6(8):129-135.

Pitout, J. D. D., Daniel, B. G., Lorraine, C., Kevin, B. L. (2009). Molecular Characteristicsof Extended-Spectrum-β-Lactamase-Producing Escherichia coli Isolates Causing Bacteremia in the Calgary Health Region from 2000 to 2007: Emergence of Clone ST131 as a Cause of Community-Acquired Infections. *Antimicrob. Agents Chemother*. 53(7): 2846 – 2851.

Roy, K., Lebens, M., Svennerholm, A., Teneberg, S. (2009). Enterotoxigenic Escherichia coli EtpA mediates adhesion between flagella and host cells *Nature* 457 – 594- 598.

Sackey, B. A., Mensah, P., Collision, E., Sakyi., Dauwson, E. (2001). Campylobacter,Salmonella, Shiegella and Escherichia coli in live and dressed poultry from metropolitan Accra. *Int.J.Food Microbiol.71:* 21-28.

Tabatabaei, R. R., Nasirian, A. (2003). Isolation, Identification and Antimicrobial Resistance Patterns of E. coli Isolated from Chicken Flocks. *Iran. J. Pharmacol.Ther* 22: 39-42.

Tivendale, K. A., Noormohammadi, A. H, Allen, J. L, Browning, G. F.(2009). The conserved portion of the putative virulence region contributes to virulence of avian pathogenic Escherichia coli. *Microbiology* 155:450-460.

Uchechi, N. E. and Erinma, K. (2007). Investigation of plasmid DNA and antibiotic resistance in some pathogenic organisms. *Afr. J. Microbiol.* 6(7): 877-880.

Uma, B., Prabhakar,K., Rajendran, S., Kavitha, K and Sarayu L. Y. (2009) Antibiotic Sensitivity and Plasmid Profiles of *Escherichia coli* Isolated from Pediatric Diarrhea. *J Glob. Infect Dis;* 1(2): 107–110.

Van der- Auwera, G. A., Krol, J. E, Suzuki, H., Foster, B., Van Houdt, R., Brown, C. J, Mergeay, M,TopEM (2009). Plasmids captured in C. metallidurans CH34: defining the PromA family of broad-host-range plasmids. Antonie van Leeuwenhoek 96:193-204.

Whitworth, J. H., Fegan, N., Keller, Gobius, J. K. S, Bono, J. L, Call, D. R., Hovde, C. J, Hancock, D. D, Besser, T. E. (2008). At an international scale, E. coliO157:H7 Stx bacteriophage insertion genotypes have a variable distribution between human and cattle hosts. *Appl. Environ. Microbiol.* 74: 7447-7450.

Yoon, J. W , Hovde, C. J. (2008). All blood, no stool: enterohemorrhagic Escherichia coliO157:H7 infection. *J. Vet. Sci.* 9:219-231

Zhang, J., Massow, A., Stanley ,M, *et al.* (2011) .Contamination rates and antimicrobial resistance in Enterococcus spp., Escherichia coli, and Salmonella Isolated from "no antibiotics added"-labeled chicken products. *Foodborne Pathog Dis.* **8**(11): 1147–115.