MOLECULAR IDENTIFICATION OF E. COLI O157:H7, VIRULENCE GENES AND QUINOLONE / FLUOROQUINOLONE RESISTANT GENES AMONG E. COLI ISOLATES FROM RETAILED MEAT IN IBADAN, SOUTHWEST NIGERIA

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ABSTRACT

Escherichia coli is classified based on their pathogenicity factor, the strain *E. coli* O157:H7 is implicated in diarrhoea leading to haemolytic uremic syndrome. Therefore this study identified *E. coli* O157:H7, virulence and quinolone/fluoroquinolone resistant genes in *Escherichia coli* isolates from meat.

Isolates of *Escherichia coli* from meat were identified by standard laboratory methods using Microbact GNB 12E (Oxoid), *E. coli* O157:H7 was identified by serotyping and the presence of virulence gene was determined by molecular methods. Antibiotic susceptibility of *E. coli* and quinolone/fluoroquinolone resistance (qnr) genes were determined using disc diffusion and molecular methods respectively.

Out of the 130 *E. coli* identified, 72 (55.4%) were resistant to at least one or more of the antibiotics tested including quinolone/fluoroquinolones. *E. coli* O157:H7 were detected serologically 5 (3.9%) and by the multiplex PCR 8 (6.2%) out of which, 2 (25%) carried eaeA, hly, rfbE and $flich_7$ genes, 1(12.5%) carries eaeA, rfbE and hly genes, 1(12.5%) carries hly, rfbE, $flich_7$ and stx2 genes, 3(37.5%) carries hly, rfbE and $flich_7$ genes, 1 (12.5%) carries rfbE and hly genes while none of the isolates have stx1 genes. Quinolone resistant genes (qnr) was harboured by 41 (56.9%) of which 5 (3.9%) and 36(27.7%) isolates carried qnrA and qnrB, respectively. Only 2 (50%) of the *E. coli* O157:H7 harbour qnrB, no qnrA was detected.

The *E. coli* isolated from meat carries virulence and qnr resistance gene which could be potential vehicles for spread of multi-drug resistant to humans.

Keywords: *E. coli* O157:H7, Quinolone resistant genes, virulence gene

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INTRODUCTION

Background

Escherichia coli is a member of the family Enterobacteriaceae found in the lower intestines of warm-blooded animals and birds although most strains of the *E. coli* are non-pathogenic, some can cause a variety of intestinal and extra-intestinal infections in men, animals, and poultry (Todar, 2007). *E. coli* strains have been classified into different pathogenicity groups, based on their virulence properties (Nataro & Kaper, 1998). Many produce a variety of potent toxins, including Shiga-like toxins (Stx) (Wang *et al*., 2002). The Shiga toxin-producing *E. coli* (STEC) isolates express virulence in humans by this toxin (Nataro & Kaper, 1998).

The most recognized representative pathotype of

STEC is the Enterohemorrhagic *E. coli* (EHEC) strain O157:H7 (Caprioli *et al*., 2005; Newell & Ragione, 2018) which causes diarrhea, haemorrhagic colitis, and haemolytic uremic syndrome in humans (García *et al.,* 2010). The E. coli O157:H7 have been associated with food and water borne infections. Pathogen has been isolated from outbreaks of foodborne infections involving comsumption of beef and beef products (Sarimehmetoglu *et al*., 2009; Jeshveen, 2012). The strain is identified by the presence of one or two these virulence genes rfbE (O157 antigen), eae (intimin), stx1 (Shiga toxin 1), stx2 (Shiga toxin 2), hlyA (hemolysin) and fliCh7 (flagellar antigen) (Nataro & Kaper, 1998; Sarimehmetoglu *et al*., 2009; Jeshveen, 2012).

Quinolones are synthetic antimicrobial agents with broad antibacterial spectrum and very potent activity against Enterobacteriaceae including *E. coli* (Chen *et al*., 2012). *E. coli* is increasingly becoming more resistant to these antibiotics and are involved in transmission of antibiotic-resistance genes to other Enterobacteriaceae in the environment (Hooper & Jacoby, 2015; Laarem *et al*., 2017).Quinolones have long been used as one of the antimicrobial agents of choice for the treatment of various Gram-negative infections both in human and in veterinary medicine ostensibly increasing the rate of resistant isolates all over the world (Andriole, 2005; Caruso *et al*., 2018). Inappropriate, unnecessary and inevitable administrations of these antibiotics in human and food animals over time have resulted in the development and spread of resistant bacteria to animals, humans and the environment via food, water, direct animal contact, and other pathways (Gorbach, 2001; Rezazadeh *et al*., 2016) thus limiting treatment options.

The rise in quinolone resistance threatens the clinical utility of this important drug which acts by converting their targets, gyrase and topoisomerase IV, into toxic enzymes that fragment the bacterial chromosome (Aldred *et al*., 2014). Quinolone resistance in Enterobacteriaceae results mainly from mutations in type II DNA topoisomerase genes and/or changes in the expression of outer membrane and efflux pumps. Plasmid-mediated resistance mechanisms also play a significant role in fluoroquinolone resistance, and is mediated by the genes (qnr) encoding proteins that belong to the pentapeptide repeat family and protect DNA gyrase and topoisomerase IV against quinolone compounds (Wang *et al*., 2008).

The three major groups of qnr determinants are qnrA, qnrB, and qnrS have been identified (Kim *et al.,* 2009, Rezazadeh *et al.,* 2016). The first plasmid-mediated quinolone-resistance gene (qnrA) was identified in a clinical strain of *Klebsiella pneumoniae* isolated in Alabama in 1998 (Mammeri, 2005). The other two determinants of qnr (qnrB and qnrS) have subsequently been observed in other enterobacteria species including *E. coli*, *Enterobacter spp., Salmonella spp*., and *Klebsiella pneumoniae* (Andres, 2013).

Much reported work in Nigeria had involved conventional identification of *E. coli* O157:H7 from different sources (Olorunshola *et al*., 2000; Itelima & Agina, 2011). This study therefore aimed at molecular identification of *E. coli* O157:H7 isolate from retailed meat samples in Ibadan, Nigeria, study of its virulence and quinolone/fluoroquinolone resistant genes.

Materials and Methods

Isolates used for the study:

The identified *E. coli* isolated from retailed meat an earlier study (Ayodele *et al.,* 2019) was reactivated by sub-culturing on MacConkey agar and Sorbitol MacConkey agar (Oxoid, UK), incubated at 37^{o}C for 18-24 hours in the incubator (Gulfex Medical and Scientific, England). The isolates were reconfirmed biochemically using Microbat 12E identification kit (Oxoid, UK) as described in the Manufacture's manual. Serological identification was carried out by agglutination method using Remel Wellcolex *E. coli* O157:H7 kit (Remel Europe Ltd, Kent UK) following manufacturer's instruction while PCR method was used for molecular identification of the *E. coli* O157:H7 isolate (Firoozeh *et al.,* 2014).

Antimicrobial Susceptibility of *E. coli*

The confirmed *E. coli* was inoculated on Mueller-Hinton agar (OXOID, UK) and antibiotic disks (Ampicillin, Amoxicillin/Clavulanic acid, Gentamicin, Cefuroxime, Ceftazidime, Meropenem Nalidixic acid, Ciprofloxacin, Pefloxacin, Norfloxacin and Levofloxacin) disc

(OXOID, UK) were placed using Kirby-Bauer disc diffusion method according to the Clinical Laboratory Standards Institute guidelines (CLSI, 2013). *E. coli* ATCC 25922 was used as control. The zones of clearing around the disc were measured and obtained data compared and interpreted as sensitive, resistant or intermediate (CLSI, 2013).

DNA extraction and qnr genes detection

Total DNA was extracted from the confirmed *E. coli* isolates using Bacteria DNA extraction kit (Jena Bioscience, Germany) following the manufacturer's instructions. The DNA was used to identify *E. coli* O157:H7 and screened for quinolone/fluoroquinolone resistance genes (qnrA and qnrB) using primers in Table 1 and 2 respectively, as described by Firoozeh *et al.,* (2014). The amplification was carried out by adding 5µL of the of DNA sample to multiplex PCR reaction master mix primers for quinolone and fluoroquinolone resistance genes containing a premix of PCR buffer, Magnesium chloride, dNTPs, and Taq polymerase enzyme in optimized concentrations (Jena Bioscience, Germany) to obtain a 25µL reaction mix. The PCR reaction mixture was put in the Thermal cycler (Master Cycler Gradient Eppendorf, Hamburg, Germany) programmed at 95°C for 5 minutes to activate the Taq polymerase enzyme followed by 35 cycles of denaturation of the double-stranded DNA at 95°C for 45 seconds, primer annealing at 51°C for 45 seconds, elongation at 72°C for 45 seconds and final extension at 72°C for 7 minutes. The electrophoresis of amplified products was performed as described by Lee *et al.,* (2012) using 2% agarose gel, stained with ethidium bromide for 15 minutes and visualized under the ultraviolet light using the Trans-illuminator (Bio-Rad, Italy).

Results

All the 130 isolates from retailed meat were confirmed as *E. coli* biochemically, the serotyping identified only 5 (3.9%) as *E. coli* O157:H7 whereas, the multiplex PCR detected 8 (6.2%). Out of the eight (8) *E. coli* O157:H7 identified by multiplex PCR assay, all 8 (100%) carried *hly* and *rfbE* genes, 6 (75%) carries $fliC_{h7}$, 3 (37.5%) carries *eaeA* while only1 (12.5%) *stx2*. None of the isolates have *stx1* gene (Figure 1).

Antibiotic susceptibility test revealed that 72 (55.4%) out of 130 *E. coli* were resistant to at least one quinolone/fluoroquinolone, 41 (56.9%) harboured qnr genes, out of which 5 (3.9%) where qnrA and 36 (27.7%) qnrB, (Figure 2 and 3). The *E. coli* O157:H7 4 (50%) isolated were resistant to one or more quinolone/fluoroquinolone. qnrB gene was detected in only 2 (25%) of the *E. coli* O157:H7 while qnrA gene was absent (Figure 4 and 5).

Discussion

The identification of *Escherichia coli* O157:H7 in this study is in line with various reports of *E. coli* O157:H7 in meat (Hiko *et al*., 2008; Olatoye, 2010; Hessain, 2015). The strain has been isolated from the intestines of healthy cattle, deer, goats, and sheep (Newell & La Ragione, 2018). Cattle are probably the most important source of *E. coli* O157:H7 infections in humans, outbreaks of which have been associated directly with consumption of processed meat product from cattle and other ruminant animals (Sarimehmetoglu *et al*., 2009; WHO, 2018).

The identified *E. coli* O157:H7 strains carried at least two of the virulence genes tested. The carriage of two more virulence genes by *E. coli* O157:H7 have been reported by similar studies (Hessain *et al*., 2015; Oloyede *et al*., 2016; Ayaz, *et al*., 2016). The most common genes identified in this study are *hlyA, rfbE and* $fliC_{h7}$. *E. coli* O157:H7 form Shiga toxin encoded by *stx1* and *stx2* genes which are the A-B type toxin that inhibits protein synthesis and causes haemorrhagic colitis and haemolytic-uremic syndrome (Jeshveen *et al.,* 2012; Javadi *et al*., 2016). Only *stx2* gene was observed in this study. The production of *Stx2* by strains of *E. coli* O157:H7 have been associated with severe human disease with an increased risk of systemic complications (Boerlin *et al.,* 1999; García *et al.,* 2010). *Stx2* gene is frequently detected in strains isolated from patients with haemolytic-uremic syndrome and uncomplicated diarrhoea (Friedrich *et al*., 2002).

All the virulence genes identified in this study are associated with severity of infection caused by the E. coli O157:H7. Haemolysin is encoded by hlyA gene which is found in almost all O157 strains, rfbE gene expresses O157 antigen (Jeshveen *et al.,* 2012). The flagella antigen encoded by $fliC_{h7}$ gene plays a vital role in bacterial movement and

distribution in host intestine and tissues (Javadi *et al*., 2016). Furthermore the eaeA gene is known to encode intimin, which is responsible for adherence of this pathogen to the intestinal lining and causing human illnesses (Sarimehmetoglu *et al*., 2009).
Quinolone/fluoroquinolone resistant genes (qnr) were detected in majority of the isolates; qnrB 27.7% was more predominant than qnrA 3.9%. qnrA and qnrB genes have been reported in animals and human in similar studies (Chen *et al*., 2012; Caruso *et al*., 2018). These resistant genes carried by bacteria can be transferred from animals to human through the food chain. The detection of quinolone/fluoroquinolone-resistant *E. coli* among the isolates from meat is of public health significance because the resistant genes may be transferred to consumers who will subsequently develop resistance to therapeutic agents.

Conclusion

This study established the existence of *E. coli* O157:H7 strains that possessed at least two of the virulence (*fliC*$_{h7}$, *rfbE, eaeA, hly* and *stx2*) genes which could predispose the consumer of the meat to food borne infection. The presence of quinolone/fluoroquinolone resistant genes (qnrA and qnrB) in the isolates makes them potential vehicles for spread of quinolone/fluoroquinolone drug resistance to humans.

Transparency declarations: None to declare

Authors' contributions

AM conceived, designed supervised the experiment and proofread the manuscript; OA designed, collected samples, performed the experiment, drafted the manuscript, AO assisted in molecular work and analysis, PA assisted in experimental design and manuscript drafting, VK assisted in experimental aspect. All authors read and approved the final manuscript.

Ethics approval and consent to participate: Not applicable

Competing interests: The authors declare that they have no competing interest

Table 1: Primers and primer sequences for the identification of *E. coli* O157: H7

Primers	Sequences (5′ - 3′)	Target gene	Amplicon size (bp)	Reference
FLICH7-F	GCGCTGTCGAGTTCTATCGAGC	fliC$_{h7}$	625	Sarimehmetoglu *et al*., 2009
FLICH7-R	CAACGGTGACTTTATCGCCATTCC			
rfbE-F	CAGGTGAAGGTGGAATGGTTGTC	rfbE	296	Jeshveen *et al.*, 2012
rfbE-R	TTAGAATTGAGACCATCCAATAAG			
SLT1-F	TGTAACTGGAAAGGTGGAGTATACA	stx$_1$	210	Sarimehmetoglu *et al*., 2009
SLT1-R	GCTATTCTGAGTCAACGAAAAATAAC			
SLT11-F	GTTTTTCTTCGGTATCCTATTCC	stx$_2$	484	Sarimehmetoglu *et al*., 2009
SLT11-R	GATGCATCTCTGGTCATTGTATTAC			
AE22	ATTACCATCCACACAGACGGT	eaeA	397	Sarimehmetoglu *et al*., 2009
AE20-2	ACAGCGTGGTTGGATCAACCT			
MFS1-F	ACGATGTGGTTTATTCTGGA	Hly	166	Sarimehmetoglu *et al*., 2009
MFS1-R	CTTCACGTCACCATACATAT			

Table 2: The primers and primer sequences for quinolone and fluoroquinolone resistance genes

Primers	Sequences (5- - -3)	Target gene	Amplicon size (bp)
qnrA F	ATTTCTCACGCCAGGATTTG	qnrA	516
qnrA R	GATCGGCAAAGGTTAGGTCA		
qnrB F	GATCGTGAAAGCCAGAAAGG	qnrB	469
qnrB R	ACGATGCCTGGTAGTTGTCC		

Firoozeh *et al.,* (2014)

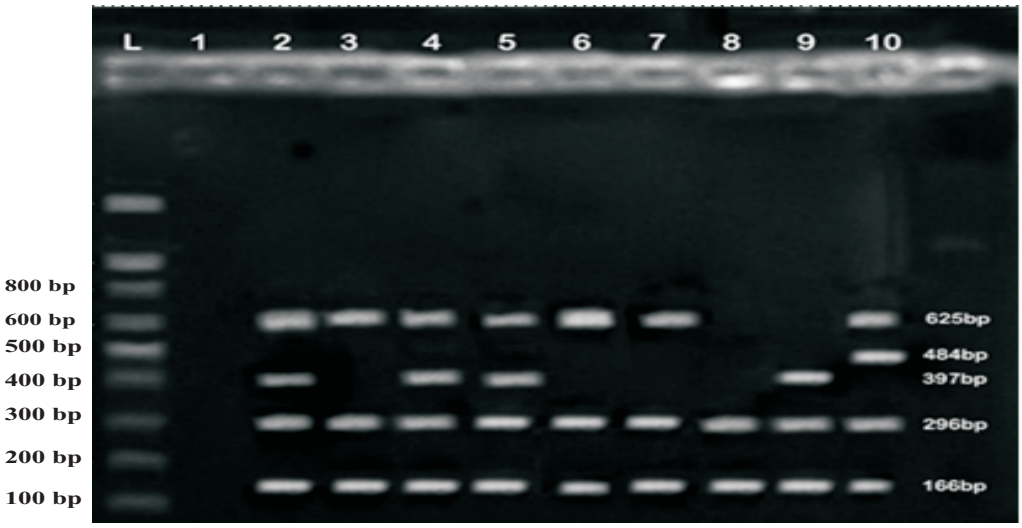

Figure 1: Gel electrophoresis of amplicons of *fliC$_{h7}$ (625bp), stx1 (210bp), stx2 (484bp), eaeA (397bp), hly (166bp)* and *rfbE (296bp)* genes of *E. coli* O157:H7 in *E. coli* isolates. Lane L: DNA ladder (100 bp), lane 1: negative control, lane 2: positive control, lanes 3 - 11: amplicons from the isolates. Lanes 3, 6, 7: *hly, rfbE, fliC$_{h7}$* genes; lanes 4, 5: *hly, rfbE, eaeA, fliC$_{h7}$* genes; lanes 8: *hly, rfbE* genes; lane 9: *hly, rfbE, eaeA*; lane 10: *hly, rfbE, stx2, fliC$_{h7}$* genes.

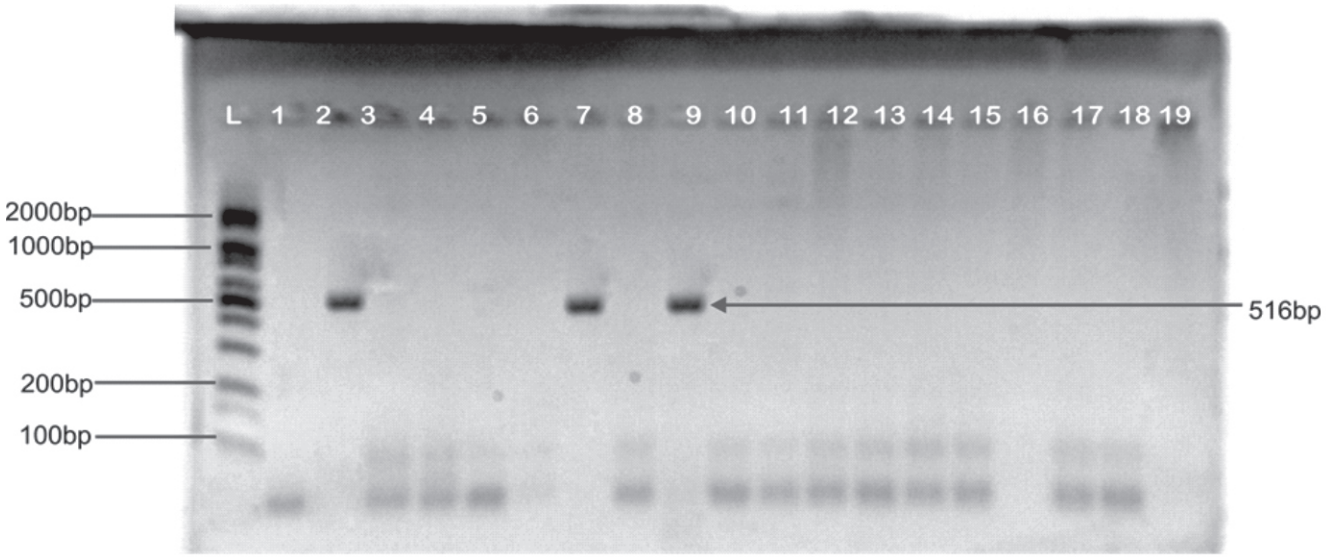

Figure 2: Gel electrophoresis of amplicons showing the presence of quinolone and fluoroquinolone resistance genes (qnrA) in *E. coli* isolates. Lane L: DNA ladder (100 bp), lane 1: negative control, lane 2: positive control and lanes 3 -19: amplicons from the isolates. Lanes 7 and 9 have qnrA genes

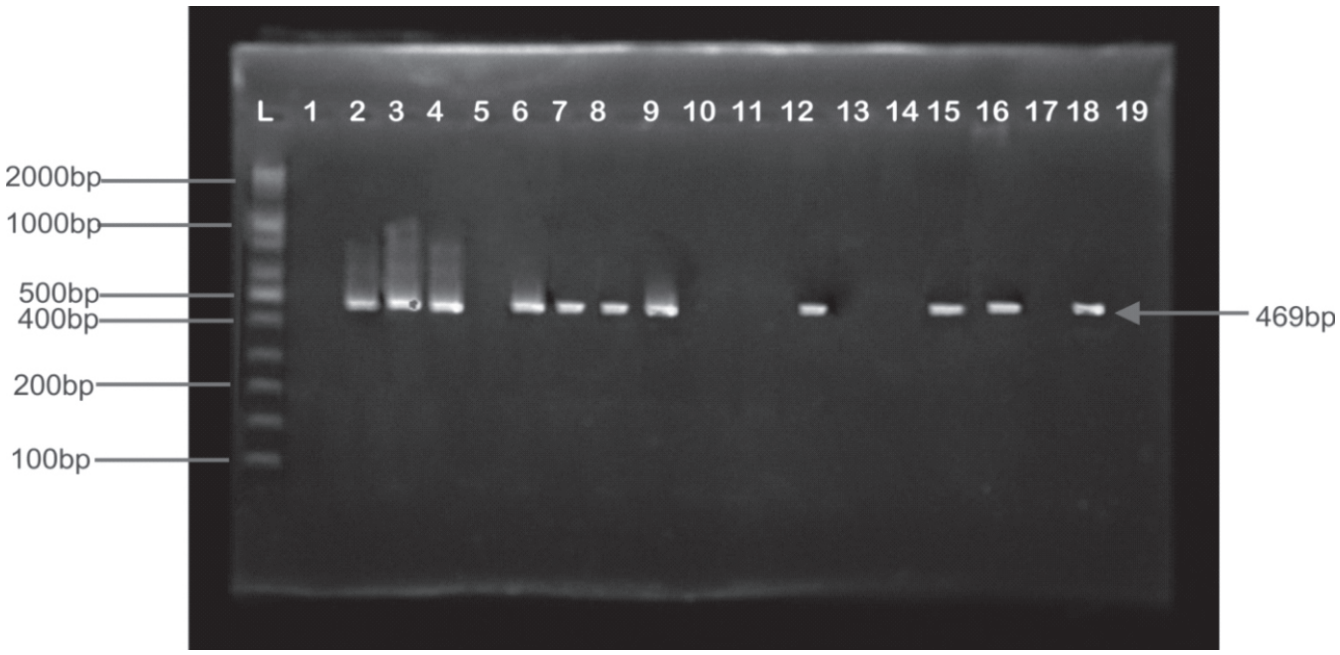

Figure 3: Gel electrophoresis of amplicons showing the presence of quinolone and fluoroquinolone resistance genes (qnrB) in *E. coli* isolates. Lane L: DNA ladder (100 bp), lane 1: negative control, lane 2: positive control and lanes 3 - 19: amplicons from the isolates

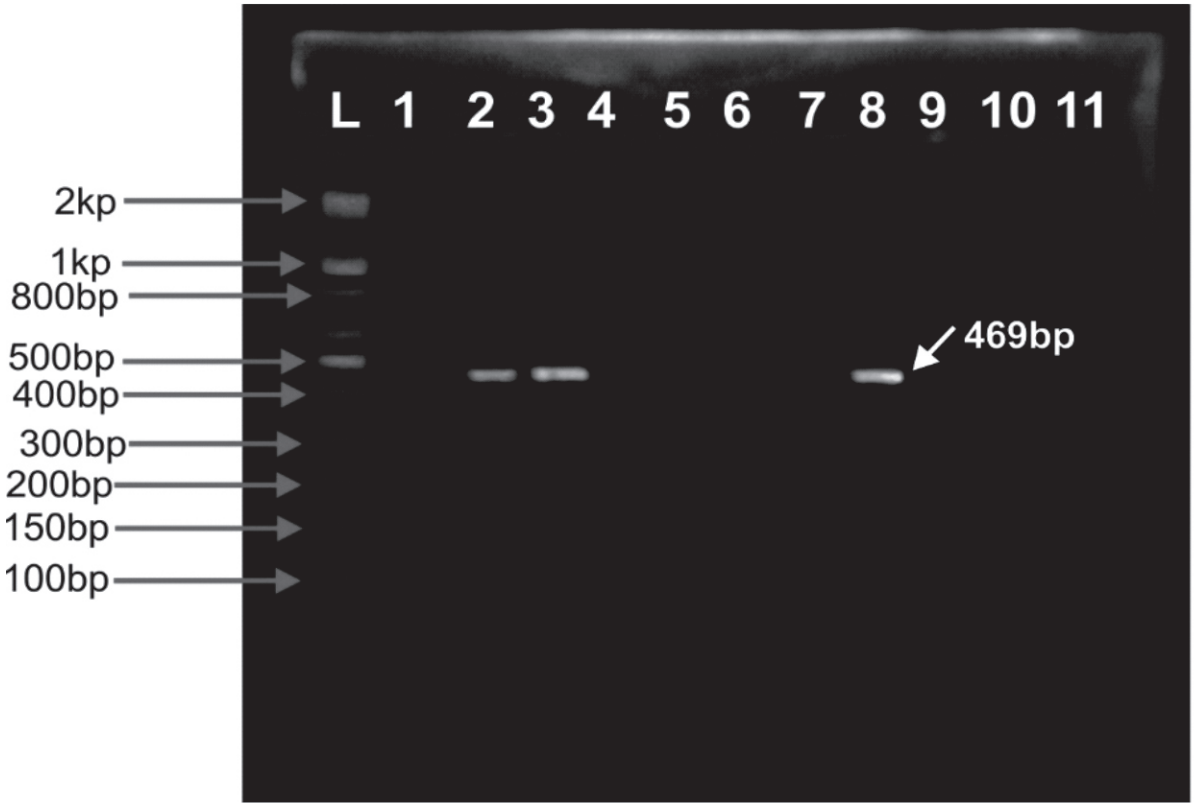

Figure 4: Gel electrophoresis of amplicons showing the presence of quinolone and fluoroquinolone resistance genes (qnrB) in *E. coli* O157:H7 isolates. Lane L: DNA ladder (100 bp), lane 1: negative control, lane 2: positive control and lanes 3 -11: amplicons from the isolates

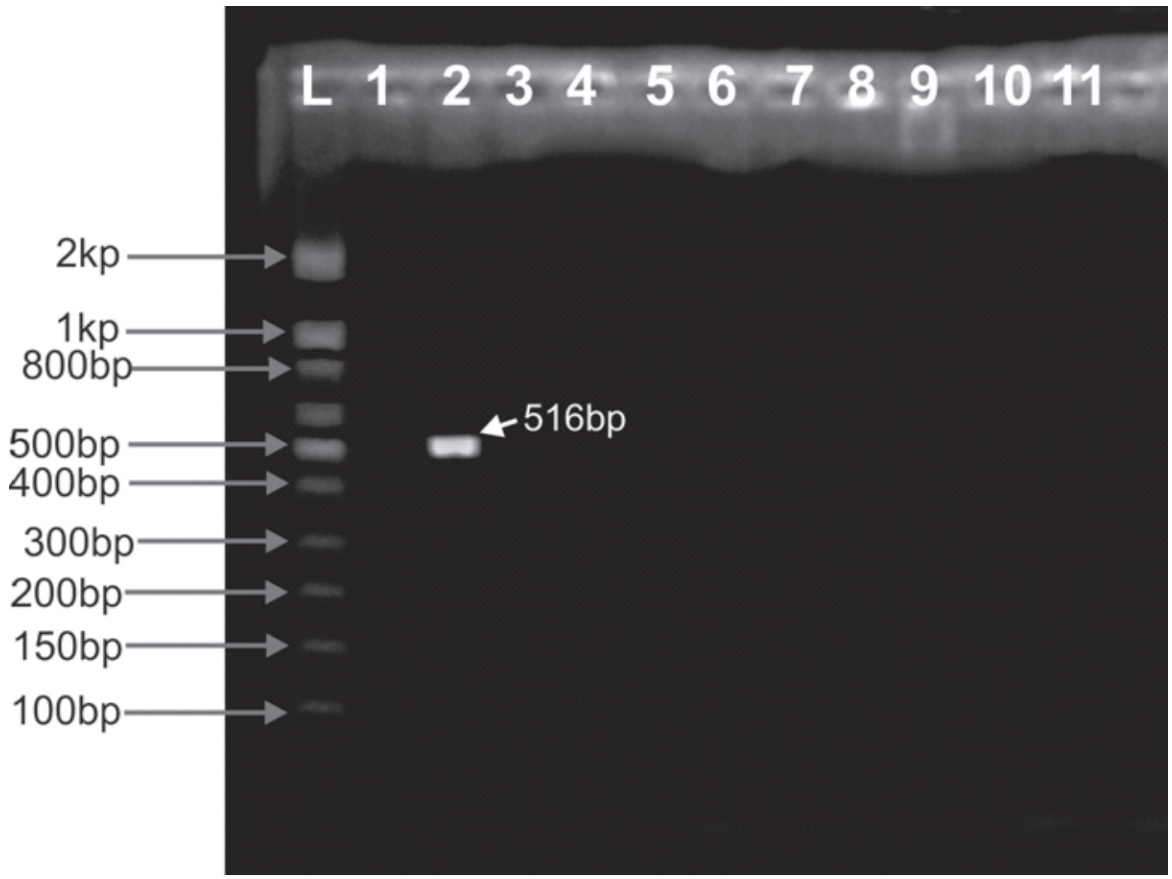

Figure 5: Gel electrophoresis of amplicons showing the absence of quinolone and fluoroquinolone resistance genes (qnrA) in *E. coli* O157:H7 isolates. Lane L: DNA ladder (100 bp), lane 1: negative control, lane 2: positive control and lanes 3 - 11: amplicons from the isolates

References

Aldred, K. J, Kerns, R. J.& Osheroff, N. (2014). Mechanism of Quinolone Action and Resistance. *Biochemistry*, 53, 1565-1574 dx.doi.org/10.1021/bi5000564.

Andres, P., Lucero, C., Soler-Bistue,´ A., Guerriero, L., Albornoz, E., Tran, T.& Zorreguieta, A., Galas, M., Corso, A., Tolmasky, M. E.& Petroni, A. (2013). Differential distribution of plasmid-mediated quinolone resistance genes in clinical enterobacteria with unusual phenotypes of quinolone susceptibility from Argentina. *Antimicrobial Agents* and *Chemotherapy*. 57(6), 2467-2475. doi: 10.1128/AAC.01615-12.

Andriole, V. T. (2005). "The quinolones: past, present, and future," *Clinical Infectious Diseases*. 41(2), S113-S119.

Ayaz, N. M., Cufaoglu, G., Ormeci, E. & Oz, B. (2016). Presence of *Staphylococcus aureus* and Shiga Toxigenic *Escherichia coli* O157:H7 in Raw Meat in Agri, Turkey. *International Journal of Enteric Pathogen*, 4(3), e36523.

Ayodele, O. A., Deji-Agboola, A. M., Akinduti, P. A. & Faneye, A. O. (2019): Phylo-diversity of prevalent human *E. coli* O157:H7 with strains from retailed meat and fish in selected markets in Ibadan Nigeria, *Journal of Immunoassay and Immunochemistry*. doi: 10.1080/15321819.2019.1694942

Boerlin P., McEwen S.A., Boerlin-Petzold F., Wilson J.B., Johnson R.P.& Gyles C.L. (1999). Associations between virulence factors of Shiga toxin-producing *Escherichia coli* and disease in humans. *Journal of Clinical Microbiology,* 37, 497– 503.)

Caprioli. A., Morabito. S., Brugère H., & Oswald, E. (2005). Enterohaemorrhagic *Escherichia coli*: emerging issues on virulence and modes of transmission. *Veterinary Research,* 36: 289–311

Caruso, G., Giammanco A., Cardamone C., Oliveri G., Mascarella C., Capra G. & Fasciana T. (2018). Extra-Intestinal Fluoroquinolone-Resistant *Escherichia coli* Strains Isolated from Meat. *BioMed Research International*, Article ID 8714975, 7 pages. Retrieved from .

Chen, X., Zhang, W., Pan, W., Yin, J., Pan, Z., Gao, S. & Jiao X. (2012). Prevalence of qnr, aac (6′)-Ib-cr, qepA, and oqxAB in *Escherichia coli* Isolates from Humans, Animals, and the Environment. *Antimicrobial Agents* and *Chemotherapy*, 56 (6), 3423-3427.

CLSI (2013). *Performance Standards for Antimicrobial Susceptibility Testing*, 23rd ed. CLSI Supplement M100-S23, 33(1). Wayne, PA: Clinical and Laboratory Standards Institute.

Firoozeh, F., Zibaei, M. & Soleimani-As, Y. **(**2014). Detection of plasmid-mediated *qnr* genes among the quinolone-resistant *Escherichia coli* isolates in Iran. *Journal of Infectious Diseases in Developing Countries*, 8(7), 818-822.

Friedrich A.W., Bielaszewska M., Zhang W.L., Pulz M., Kuczius T., Ammon A.& Karch H. (2002) *Escherichia coli* harboring Shiga toxin 2 gene variants: frequency and association with clinical symptoms, *Journal of Infectious Diseases,* 185(1), 74– 84.

García, A., Fox, J. G. & Besser,T. E. (2010). Zoonotic Enterohemorrhagic *Escherichia coli*: A One Health Perspective *ILAR Journal*, 51(3), 221-232

Gorbach, S.L. Antimicrobial use in animal feed-time to stop. (2001) *New England Journal of Medicine*, 345, 1202-1203.

Hessain, A. M., Al-Arfaj, A. A., Zakri, A. M., El-Jakee, J. K., Al-Zogibi, O. G., Hemeg, H.A.& Saudi, I. M. I. (2015). Molecular characterization of *Escherichia coli* O157:H7 recovered from meat and meat products relevant to human health in Riyadh, SaudiArabia. *Journal of Biosciences,* 22, 725-729.

Hiko, A., Asrat, D.& Zewde, G. (2008): Occurrence of *Escherichia coli* O157:H7 in retail raw meat products in Ethiopia. *Journal of Infectious diseases in Developing Countries,* 5: 389-393.

Hooper D. C & Jacoby G. A. (2015). Mechanisms of drug resistance: quinolone resistance. *Annals of New York Academy of Science*, 17: 12830.

Itelima, J. U., & Agina S. E. (2011). The Occurrence of *Escherichia coli* O157:H7 in Market and Abattoir Meat in Plateau State, Nigeria. *Global Journal of Environmental Sciences*, 10 (1&2), 47-55

Javadi, M., Oloomi, M., & Bouzari M. S. (2016). Genotype Cluster Analysis in Pathogenic *Escherichia coli* Isolates Producing Different CDT Types. *Journal of Pathogens*, Article ID 9237127, 8 pages *Retrieved from* , on 28, February 2019

Jeshveen, S. S., Chai, L. C., Pui, C. F. & Son, R. (2012). Optimization of multiplex PCR conditions for rapid detection of *Escherichia coli* O157:H7 virulence genes. *International Food Research Journal* 19(2), 461-466.

Kim, H. B., Park, C. H., Kim, C. J., Kim, E-C., Jacoby, G. A., &Hooper, D. C. (2009). Prevalence of plasmid-mediated quinolone resistance determinants over a 9-year period. *Antimicrobial Agents and Chemotherapy*, 53(2):639-645.

Laarem, M., Barguigua, A., Nayme, K., Akila, A., Zerouali, K., El Mdaghri, N. & Timinouni, M. (2017). Occurrence of plasmid-mediated quinolone resistance and virulence genes in avian *Escherichia coli* isolates from Algeria. *Journal of Infectious diseases in Developing Countries,* 11(2), 143-151.

Lee, P.Y., Costumbrado, J., Hsu, C.Y. &Kim, Y.H. (2012).Agarose Gel Electrophoresis for the Separation of DNA Fragments. *Journal of Visual Experiment*. (62): e3923, doi:10.3791/3923.

Mammeri, H., Van De Loo, M., Poirel, L., Martinez- Martinez, L. & Nordmann, P. (2005). Emergence of plasmid-mediated quinolone resistance in *Escherichia coli* in Europe. *Antimicrobial Agents Chemotherapy*, 49(1),71-76.

Nataro, J. P., and Kaper, J. B. (1998). Diarrheagenic *Escherichia coli. Clinical Microbiology Reviews,* 11, 142-201.

Newell D. G. & La Ragione, R. M. (2018). Enterohaemorrhagic and other Shiga toxin-producing *Escherichia coli* (STEC): Where are we now regarding diagnostics and control strategies? *Transbound Emerging Diseases*.

65 (Suppl. 1), 49–71. DOI: 10.1111/tbed.12789

Olatoye, I. O. (2010). The incidence and antibiotics susceptibility of *Escherichia coli*O157:H7 from beef in Ibadan Municipal, Nigeria. *African Journal of Biotechechnology*, 9(8), 1196-1199.

Olorunshola, I. D., Smith, S. I., Coker, A. O. (2000). Prevalence of EHEC O157:H7 in patients with diarrhoea in Lagos, Nigeria. *Acta pathologica, microbiologica et immunologica Scandinavian*, 108(11), 761-763.

Oloyede, A. R., Afolabi, O. R. & Olalowo, O. S (2016). Molecular detection of virulence genes and antibiotic resistance patterns of *Escherichia coli* O157:H7 isolated from raw beef sold in Abeokuta, South-West Nigeria. *Nigerian Journal of Biotechnology,* 31, 15-21.

Rezazadeh, M., Baghchesaraei, H. & Peymani, A. (2016). Plasmid-Mediated Quinolone-Resistance (qnr) Genes in Clinical Isolates of *Escherichia coli* collected from Several Hospitals of Qazvin and Zanjan Provinces, Iran. *Osong Public Health* and *Research Perspectives*, 7(5), 307-312.

Sarimehmetoglu, B., Aksoy, M. H., Ayaz, N. D., Ayaz, Y., Kuplulu, O. & Kaplan, Y. Z. (2009). Detection of *Escherichia coli* O157:H7 in ground beef using immunomagnetic separation and multiplex PCR. *Food Control*, 20, 357-361.

Todar K. Pathogenic *E. coli* Online *Textbook for Bacteriology*. (2007). *Retrieved from* . 28, February 2019

Wang, G., Clark, C. G. & Rodgers, F. G. (2002). Detection in *Escherichia coli* of the Genes Encoding the Major Virulence Factors, the Genes Defining the O157:H7 Serotype, and Components of the Type 2 Shiga Toxin Family by Multiplex PCR. *Journal of Clinical Microbiology*, 40 (10), 3613–3619. DOI: 10.1128/JCM.40.10.3613–3619.2002

Wang, A., Yang, Y., Lu, Q., Wang, Y., Chen, Y., Deng, L., Ding, H., Deng, Q., Zhang, H., Wang, C., Liu, L., Xu X, Wang L & Shen X. (2008). Presence of qnr gene in *Escherichia coli* and *Klebsiella pneumoniae* resistant to ciprofloxacin isolated from pediatric patients in China. *BMC Infectious Diseases*, 8, 68.

WHO (2018). *E. coli.*https://www.who.int/news-room/fact-sheets/detail/e-coli. Retrieved 28, February 2019