Prevalence and Antibiogram of *Escherichia coli* O26 in Raw Milk from Nomadic Fulani Cattle in Oyo State, Nigeria

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Abstract- Escherichia coli* O26 pose health risk to humans; it has been responsible for several outbreaks across the world, with associated cases of Haemolytic Uraemic Syndrome and death. Many studies in Nigeria have focused on *E. coli* O157:H7 in milk production chain, with paucity of research on other Shiga toxin *E. coli*. This study was therefore designed to investigate the occurrence and antibiogram of *E. coli* O26 in raw milk from nomadic Fulani cattle in Oyo State, Nigeria. Using a purposive sampling method, a total of 150 pooled raw milk samples, 50 each, were collected from 3 nomadic Fulani settlements. Cefixime Tellurite Rhamnose MacConkey agar was used for isolation and *E. coli* O26 monovalent antiserum for confirmation; antibiotics susceptibility was determined and interpreted using the CLSI standard, 2015. Data were analysed using Chi Square test at $p \leq 0.05$. *E. coli* O26 was confirmed in 20 samples (13.3%). These *E. coli* O26 isolates were highly resistance, 100%, to β-lactams (Meropenem, Cefuroxime, Ceftriaxone, Cefotaxime, and Ceftazidime) and also, 95% resistance to Tetracycline but were highly susceptible to Amikacin (95%), Gentamycin (90%), Ciprofloxacin (85%) and Chloramphenicol (85%), with a lower susceptibility to Cotrimoxazole (45%). The presence of *E. coli* O26 in sampled raw milk was established. Total resistance to β-lactams and high susceptibility to Ciprofloxacin may not be clinically relevant as these groups of antimicrobials have been reported to increase Stx-production. On the other hand, amikacin, gentamicin, and chloramphenicol may be considered for *E. coli* O26 therapy but there is need for characterization of their clinical relevance for *E. coli* O26 outbreaks *in-vivo.

Keywords: **Antimicrobial resistance, *E. coli* O26, Milk**

I. INTRODUCTION

Escherichia coli O26 strains were first described by Orskov in 1951, who associated them with infantile diarrhoea and white scours. Since then, the O26 serogroup has become one of the most important groups among the enteropathogenic *Escherichia coli* (EPEC) O serogroups (Levine, 1987). Like other EPEC O serogroups, O26 includes several O:H types that differ in virulence properties (Trabulsi *et al.,* 1996). The most common O26 strains belong to serotypes O26:H11 and O26:H32, or are non-motile (O26:H3). The *eae* (EPEC attaching and effacing) gene is frequently present in O26:H11 and H3 strains, while O26:H32 strains do not possess this gene or any other enteropathogenicity marker (Saridakis *et al*., 1994). Unlike other EPEC serotypes, some O26:H11 and O26:H3 strains produce Shiga toxin (Stx). These strains have been isolated in several countries from patients with associated diarrhea, bloody diarrhea, and hemolytic uremic syndrome (HUS). Although *E. coli* O157:H7 is the predominant cause of HUS worldwide (Tarr, Gordon & Chandler, 2005). EHEC O26 can cause disease that is as severe as that caused by EHEC O157:H7 (Gerber *et al*., 2002), and there appears to be no significant difference in the long-term outcome of HUS caused by non-O157 EHEC (including EHEC O26) and EHEC O157 (Rosales Hofer & Zimmerhackl, 2012). Recent *E. coli* O26 outbreaks around the world have been associated different food sources; Outbreaks in the USA have been linked to; raw clover sprouts in 2012; Chipotle Mexican grill in 2015; flour in 2016, ground beef in 2018, flour in 2019, (CDC); and also dairy product in Romania 2016 (Peron *et al.,* 2016).

E. coli O26 is one the multi-drug resistance shiga-toxin *E. coli* (MDR STEC) organisms that is ESBL producing; Carbapenemase-producing (CP), bla_{VIM} , bla_{TEM}, $bla_{\text{CTX-M1}}$ and $bla_{\text{OXA-1}}$ genes have been reported (Elmonir *et al*., 2021). *E. coli* O26 has been reported with resistance to varying group of antibiotics; isolates from cattle has been reported with highest resistance to ampicillin, streptomycin, and tetracycline (Lee, 2009; Sasaki *et al.*, 2011; Kamara et al., 2019), similar resistance to the same antibiotics were observed in *E. coli* O26 from pigs (Chinwe *et al*., 2013), and diarrheic humans (Day *et al*., 2017). Other studies have reported resistance to penicillin, gentamicin, (Momtaz *et al.* 2013) and trimethoprim/sulphonamide (Day *et al.*, 2017). On the other hand, this organism has been reported to be susceptible to ciprofloxacin, ofloxacin, norfloxacin, ceftriaxone, amikacin, imipenem, meropenem and chloramphenicol (Lee 2009; Chinwe *et al.*, 2013; Pan *et al*., 2021)

Many studies in Nigeria have focused on *E. coli* O157:H7 in milk production chain (Adetunji and Arigbede 2011), but little has been done about other STEC serotypes. There is also paucity of research on *E. coli* O26 in food products in Nigeria. This study therefore determined the occurrence of *E. coli* O26 contamination in raw milk as well as the antibiotics susceptibility and multi-drug resistance pattern of the organism.

II. MATERIALS AND METHOD

A. Collection of Sample

Three nomadic Fulani settlements located in Iseyin, Fashola and Alaga areas of Oyo State, Southwest Nigeria were used for this study. Sample size was determined as described by Thrusfield, 2005, using an expected prevalence of 10.8%, in raw milk from North East, Nigeria (Moses *et al*., 2010) and an absolute error of 5%, the sample size calculated = 148, approximately = 150 samples.

One hundred and fifty (150) pooled raw milk samples derived from cattle within these settlements were collected (50 samples each per settlement). Samples (5mls each) were collected in sterile screw-capped sample bottles; they were collected in batches on 3 different occasions covering one settlement at a time and transported on ice to the laboratory for microbial analysis.

B. E. coli O26 Identification and Isolation from Raw Milk Samples

E. coli O26 was isolated and identified according to Reiji et al., 2002. Based on *E. coli* O26 sugar fermenting ability (non-rhamnose fermenters), Cefixime Tellurite Rhamnose Mac Conkey Agar (CTRMAC) was used for microbial culture. CTRMAC media was prepared by mixing MacConkey agar base (HiMedia, India) and rhamnose sugar (Glentham Life Science, UK) (10 g/litre) in 1 liter of distilled water and autoclaved. Potassium tellurite (2.5 mg/liter) and Cefixime (0.05 mg/liter) (Oxoid, England) were added to the autoclaved media after it has cooled to about 50˚C. Media was poured into sterile petri dishes and allowed to solidify (Reiji *et al*., 2002). 0.1ml of serially diluted (10^{-3}) raw milk was inoculated by surface plating method onto CTRMAC and incubated at 37ºC for 24 hours (10^{-3} dilution was used for culture to allow for growth of distinct colonies as proven by an initial pilot study using the raw milk samples).

Rhamnose-negative (colourless) colonies on the cultured CTRMAC were sub-cultured on freshly prepared CTRMAC. The purified isolates were used for further analyses; Gram staining, catalase test, indole test, and triple sugar iron test were performed for *E. coli* identification. Serological slide agglutination test using *E. coli* O26 monovalent antiserum (Denka Seiken, Japan) was used for confirmation (according to manufacturer's instruction).

C. Antibiotics Sensitivity Testing

E. coli O26 isolates were tested for their susceptibility to antimicrobial agents using the agar disc-diffusion method (CLSI, 2015). Commercially available Gram negative antibiotics multi-disc from Biomark Laboratory, India, was used; comprising of Tetracycline (10µg), Cotrimoxazole (25µg), Gentamycin (10µg), Cefuroxime (30µg), Chloramphenicol (10µg), Ceftriaxone (30µg), Cefotaxime (30µg), Ciprofloxacin (5µg), Amikacin (30µg), Ceftazidime (30µg), and Meropenem (10µg). The antibiotic discs were placed on nutrient agar plates previously seeded with an 18-24hrs culture of the test organisms using cotton swab and incubated at 37^{o}C for 24hrs, after which zones of inhibition were examined and interpreted accordingly.

D. Statistical Analysis

The statistical software, Open Epi toolkit (2007) was used for data analysis. The significant difference in the prevalence of *E. coli* O26 among the three settlements was defined at $p \leq 0.05$ using Chi Square Test.

E. Ethical Approval

Ethical approval was sorted from the Animal Care and Use Research Committee (ACUREC), University of Ibadan, Nigeria.

III. RESULTS

A. Prevalence of E. coli O26 in Raw Milk

Out of the 150 raw milk samples collected, 20 (13.3%) (95% CI=8.8-19.7%) were positive for *E. coli* O26. From the 50 samples collected from each of the nomadic settlements; 6 (12%) samples from Iseyin, 9 (18%) from Fashola and 5 (10%) from Alaga were positive as shown in Table 1. There was no statistically significant difference ($p= 0.47$) in the prevalence of *E. coli* O26 among the 3 settlemeents ($p>0.05$).

B. Antibiogram of E. coli O26 Isolates

The antibiotic susceptibility profile of *E. coli* O26 isolates to the 11 antibiotics is shown in Table 2. None of the antibiotics tested had 100% activity against all the isolates. The isolates showed high resistance to seven (58.3%) of the antibiotics tested; particularly 100% resistance to β-lactams group [Carbapenem (Meropenem) and Cephalosporin (Cefuroxime, Ceftriaxone, Cefotaxime, Ceftazidime)] and 95% resistance to Tetracycline, but with a lower susceptibility to Cotrimoxazole (45%) while it was highly susceptible to the Aminoglycoside group [Amikacin (95%) and Gentamycin (90%)], Ciprofloxacin (85%) and Chloramphenicol (85%) in descending order (Figure 1 and 2).

Each *E. coli* O26 isolates showed resistance to more than one antimicrobial agent. There was highest (100%) multi-drug resistance phenotype observed in cefuroxime-ceftriaxone-cefotaxime-ceftriazone-meropenem antibiotics, while lowest (5%) multi-drug resistance was found in cefuroxime-ceftriaxone-cefotaxime-ceftriazone-meropenem-amikacin antibiotics (Table 3). The cumulative effect of antibiotics against *E. coli* O26 isolates as obtained in this study is Amikacin>Gentamicin>Ciprofloxacin=Chloramphenicol>Cotrimoxazole.

Table 1: Prevalence of *E. coli* O26 in Raw Milk Samples from Various Nomadic Fulani Settlements

Sample source (Nomadic Fulani Settlement)	Number of samples		95% confidence interval
	Examined	Positive (%)	
Iseyin	50	6 (12%)	5.6-23.8
Fashola	50	9 (18%)	9.8-30.8
Alaga	50	5 (10%)	4.3-21.4
Total	**150**	**20(13.3%)**	**8.8-19.7**

Table 2 Frequency of Antibiotics Susceptibility for *E. coli* O26

S/N	Antimicrobial class	Antimicrobial agent	Disk Potency	Number of Isolates, T=20		
				Sensitive [n (%)]	Intermediate [n (%)]	Resistance [n (%)]
1.	Tetracyclines	Tetracycline	30µg	0 (0.0)	1 (5)	19 (95)
2.	Carbapenem	Meropenem	10µg	0 (0.0)	0 (0.0)	20 (100)
3.	Cephalosporins (2nd Generation)	Cefuroxime	30µg	0 (0.0)	0 (0.0)	20 (100)
4.	Cephalosporins (3rd Generartion)	Ceftriaxone	30µg	0 (0.0)	0 (0.0)	20 (100)
5.		Cefotaxime	30µg	0 (0.0)	0 (0.0)	20 (100)
6.		Ceftazidime	30µg	0 (0.0)	0 (0.0)	20 (100)
7.	Potentiated Sulphonamide	Cotrimoxazole	1.25/23.75µg	9 (45)	8 (40)	3 (15)
8.	Aminoglycoside	Amikacin	30µg	19 (95)	0 (0.0)	1 (5)
9.		Gentamycin	10µg	18 (90)	2 (10)	0 (0.0)
10.	Floroquinolone	Ciprofloxacin	5µg	17 (85%)	3 (15%)	0 (0.0)
11.	Phenicol	Chloramphenicol	30µg	17 (85%)	3 (15%)	0 (0.0)

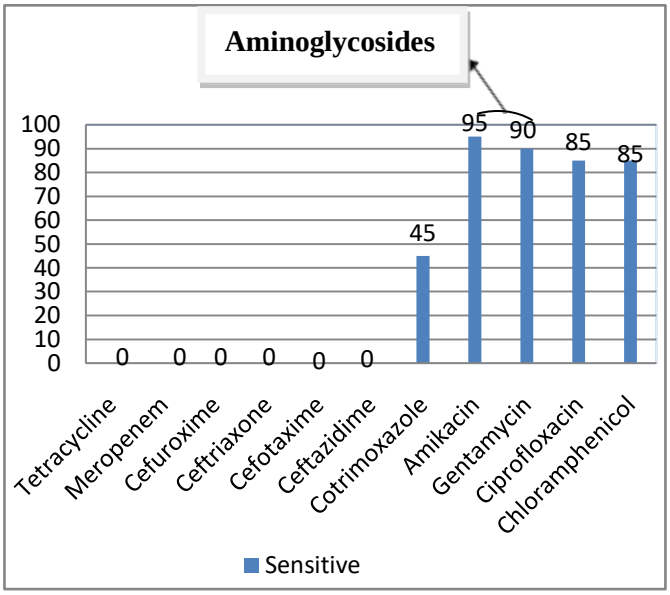

Figure 1: Antibiotics sensitivity pattern for E. coli O26; showing highest sensitivity to Aminoglycosides

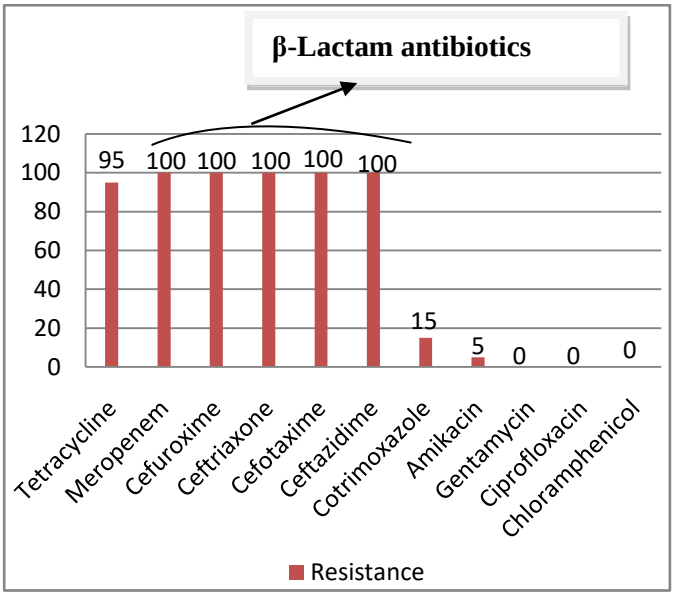

Figure 2: Antibiotics resistance pattern for E. coli O26; Showing 100% resistance to β-lactam antibiotics

Table 3 Antimicrobial Multi-Drug Resistance Pattern for *E.coli* O26

Resistance Antibiotics	Number of resistance isolates (%)
CRX-CTR-CTX-CFZ-MEM	20 (100)
CRX-CTR-CTX-CFZ-MEM-TET	19 (95)
CRX-CTR-CTX-CFZ-MEM-TET-COT	3 (15)
CRX-CTR-CTX-CFZ-MEM-TET-COT-AMK	1 (5)

Key: TET– Tetracycline MEM– Meropeneme CRX-Cefuroxime CTR– Ceftriaxone CTX– Cefotaxime CFZ– Ceftazidime COT – Cotrimoxazole AMK– Amikacin

IV. DISCUSSION

This study revealed 13.3% prevalence of *E. coli* O26 in raw milk samples from nomadic cattle which shows high public health risk to consumers of such milk without pasteurization. In previous report from Northeast Nigeria, *E. coli* O26 was found in 10.8% of the raw milk samples (Moses, Udo, Bassey & Egwu, 2010) while a higher, 37.5%, prevalence was reported in raw milk from Isfahan Province, Iran (Reza et al., 2018). Numerous factors are likely to contribute to the seemingly high prevalence of this STEC organism in raw milk which ranges from the animal management practices of the dairy farmers to the hygiene level of the milking processes. *E. coli* O26 has been isolated from both healthy and diarrheic animals, moreover, cattle has been incriminated as a major reservoir (Frohlicher et al., 2008). Milking equipment, storage and holding facility are also major sources of STEC contamination in raw milk (Anniina et al., 2019). Highest occurrence of *E. coli* O26 (18%) was observed in raw milk from Fashola milk collection centre which could be due to clusters of more dairy farms in this settlement.

β-Lactam antibiotics, especially expanded-spectrum β-lactams, such as cefotaxime, ceftazidime, ceftriaxone, and cefoperazone, has been described as one of the most clinically useful antibiotics because they combine safety with high potency against gram-negative bacteria, such as members of the family *Enterobacteriaceae*, including *E. coli*. Accordingly, expanded spectrum β-lactams are one of the groups of antibiotics recommended for the treatment of serious *E. coli* infections (Russo, 2001). In contrast, this study found 100% resistance of *E. coli* O26 to β-lactams antimicrobial group; Carbapenem (Meropenem) and Cephalosporin (Cefuroxime, Ceftriaxone, Cefotaxime, Ceftazidime), thus showing an extended spectrum β-lactam resistance. *E. coli* O26 was reported, for the first time, as an Extended-Spectrum β-lactamase (ESBL) producing STEC strain in 2005 (Yoshikazau et al., 2005) and also recently reported as one of the MDR STEC that is ESBL producing with Carbapenemase-producing (CP), bla_{VIM} , bla_{TEM}, $bla_{\text{CTX-M1}}$ and $bla_{\text{OXA-1}}$ genes (Elmonir *et al*., 2021). Furthermore, 95% resistance to tetracycline was observed, which agrees with the findings of other studies on dairy products (Natalia et al., 2014); high presence of tetracycline resistance gene (tetA, 83.3% and tetB, 100%) in *E. coli* O26 strains was reported from fermented dairy products in Iran (Farhad, Farshad, Jalal & Yousef, 2014). Highest (100%) antimicrobial multi-drug resistance phenotype was observed in cefuroxime-ceftriaxone-cefotaxime-ceftriazone-meropen-em antibiotics, which may be due to genetic resistance to this group of antibiotics by the organism. However, highest sensitivity was observed with the aminoglycoside group of anitibiotics; amikacin and gentamicin, followed by ciprofloxacin and chloramphenicol in descending order, this agrees with the findings of a study where *E. coli* O26 was reported to be sensitive to amikacin (>90%), gentamicin (80%), ciprofloxacin (100%) and chloramphenicol (80%) (Chinwe et al., 2017).

However, there have been concerns that use of antimicrobials for treatment of STEC infection may result increase Shiga toxin production and release thus increasing the risk of HUS development (Kakoullis *et al*., 2019), and therefore, their use has been contraindicated. However, not all studies were able to confirm Stx induction or an increase in the amount of HUS incidences in response to antibiotics. The effects of antibiotics on *stx* expression vary greatly and are dependent on the antibiotic class, the antibiotic concentration, the respective STEC strain, as well as the Stx subtype (Nassar *et al*. 2013). While the results obtained from some antibiotic classes, such as β-lactams, are conflicting, ansamycins (rifampicin) and chloramphenicol consistently yielded promising results in *in vitro* studies while fluoroquinolones were regularly associated with toxin induction. Use of antibiotics that inihibit translation or translation, such as rifampicin supplied in advance or simultaneously with antibiotics such as ciprofloxacin have been shown to efficiently clear the bacteria (Muhlen et al., 2020; Muhlen & Dersh, 2020). Unlike the common serotype O157:H7, STEC O104:H4 does not release STX in response to therapeutic concentrations of ciprofloxacin, meropenem, fosfomycin, and chloramphenicol (Corogeanu *et al.*, 2012). Some of the antibiotics proposed for urgent therapeutic or prophylactic use during the EHEC O104:H4 outbreak, specifically meropenem, azithromycin, and rifaximin, did not induce stx_2-harboring phages or increase stx_2 transcription or Stx2 production in the outbreak isolates in an *in vitro* system. Indeed, these antibiotics, regularly at 1/2 MIC but also at lower concentrations, significantly decreased one or more of these processes (Martina *et al.*, 2012). Therefore, it is important that the response of the respective STEC strain to antibiotics should be rapidly characterized in order to identify antibiotics that do or do not enhance the release of STX. This will eventually allow clinical studies tackling the question

whether antibiotic treatment impacts on the eradication of STEC, clinical course of disease, and frequency of carriers (Corogeanu *et al*., 2012).

V. CONCLUSION

This study detected the presence of *E. coli* O26, a pathogenic STEC strain, in raw milk, which affirms the pending danger in the consumption of such milk or it products unpasteurized. Also, the study revealed 100% multidrug resistance to β-lactams, even though these groups of drugs are not currently considered for treatment of STEC infections because of their possible contribution to Stx-production and transfer. *E. coli* O26 from this study was susceptible to amikacin, gentamicin, ciprofloxacin, chloramphenicol, and cotrimoxazole; however, several studies have confirmed the contribution of ciprofloxacin to increase Stx-production. There is need for specific characterization of *E. coli* O26 response to various antibiotics in order to identify antibiotics that do not increase Stx-production that can be relevant for treatment during outbreak.

REFERENCES

[1] Adetuniji, V.O. & Arigbede, M.I., (2011); Occurrence of *E. coli* O157:H7 and *Listeria monocytogenes* and Identification of HACCP in production operations of a typical tropic cheese "Wara" and Yoghurt. Pakistan Journal of Nutrition 10 (8).

[2] Anniina, J., Hanna, C., Saija, H., Jukka, R., Mirko, R., Joana, I., Miia, L. & Marjaana, H., (2019). Longitudinal Study of Shiga Toxin-Producing *Escherichia coli* and *Campylobacter jejuni* on Finnish Dairy Farms and in Raw Milk. Applied Environmental Microbiology 85:e02910-18

[3] Carl, M.S., Jianghong, M. & David, G.W., (2003). Antimicrobial Resistance of *Escherichia coli* O26, O103, O111, O128, and O145 from Animals and Humans. Emerging Infectious Diseases. 2003 January; 9(1): 140.

[4] CDC, report of selected E. coli Outbreaks https ://www.cdc.gov/ecoli/outbreaks.html (retrieved on March 16th, 2021).

[5] Chinwe, J.I., Ishmael, F.J., Benson, C.I., Larry, C.O. & Anthony I.O., (2017). Antibiotic resistance profiles of Escherichia coli O26, O145, and O157:H7 isolated from swine in the Eastern Cape Province, South Africa. Asian Pacific Journal of Tropical Disease 7.2017D7-9.

[6] CLSI Performance Standards for Antimicrobial Disk Susceptibility Testing, (2015). Twenty-fifth Informational supplement;CLSI document M100-25. Wayne, PA: Clinical and Laboratory Standards Institute; January 2015.

[7] Corogeanu, D., Willmes, R., Wolke, M. *et al.* Therapeutic concentrations of antibiotics inhibit Shiga toxin release from enterohemorrhagic *E. coli* O104:H4 from the 2011 German outbreak. BMC Microbiol 12, 160 (2012).

[8] Day, M., Doumith M., Jenkins, C., Dallman, T.J., Hopkins, K.L., Elson, R., Godbole, G. and Woodford N. (2015). Antimicrobial resistance in Shiga toxin-producing *Escherichia coli* serogroups O157 and O26 isolated from human cases of diarrhoeal disease in England, 2015. Journal of Antimicrobial Chemotherapy, Volume 72, Issue 1, January 2017, Pages 145–152.

[9] Elmonir, W., Shalaan, S., Tahoun, A. *et al.* (2021). Prevalence, antimicrobial resistance, and genotyping of Shiga toxin-producing *Escherichia coli* in foods of cattle origin, diarrheic cattle, and diarrheic humans in Egypt. Gut Pathog 13, 8 (2021).

[10] Farhad, S.D., Farshad, Y., Jalal, M. & Yousef, V., (2014). Virulence factors, serogroups and antimicrobial resistance properties of *Escherichia coli* strains in fermented dairy products; Biomedical Central Research Notes volume 7, Article number: 217 (2014).

[11] Frohlicher, E., Krause, G., Zweifel, C., Beutin, L. & Stephan, R., (2008). Characterization of attaching and effacing *Escherichia coli* (AEEC) isolated from pigs and sheep. Biomedical central Microbiology 8:144.

[12] Glenn, B., Pierre, B., Youri, G., Sabine, L., & Denis, P., (2010). Antimicrobial Resistance Testing of Verocytotoxin-Producing *Escherichia coli* and First Description of TEM-52 Extended-Spectrum β-Lactamase in Serogroup O26. American Society of Microbiology.

[13] Kakoullis, L.., Papachristodoulou, E., Chra, P., and Panos, G. (2019). shiga toxin-induced haemolytic uraemic syndrome and the role of antibiotics: a global overview. J. Infect. 79, 75–94.

[14] Karama, M., Mainga, A.O., Cenci-Goga, B.T. *et al.* (2009). Molecular profiling and antimicrobial resistance of Shiga toxin-producing *Escherichia coli* O26, O45, O103, O121, O145 and O157 isolates from cattle on cow-calf operations in South Africa. Science Report 9, 11930 (2019).

[15] Lee, J.H., (2009). Antimicrobial resistance of *Escherichia coli* O26 and O111 isolates from cattle and their characteristics. Veterinary Microbiology Volume 135, Issues 3–4, 30 March 2009, Pages 401-405

[16] Levine, M.M., (1987). *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic enteroinvasive, enterohemorrhagic, and enteroadherent. Journal of Infectious Diseases. 155, 377-390.

[17] Martina, B., Evgeny, A.I., Wenlan, Z., Andreas, B., Frieder, S., Alexander, M., Georg, P., and Helge, K. (2012). Effects of Antibiotics on Shiga Toxin 2 Production and Bacteriophage Induction by Epidemic *Escherichia coli* O104:H4 Strain. Antimicrob Agents Chemother. 2012 Jun; 56(6): 3277–3282.

[18] Momtaz, H., Dehkordi, F.S., Hosseini, M.J. *et al.* (2013). Serogroups, virulence genes and antibiotic resistance in Shiga toxin-producing *Escherichia coli* isolated from diarrheic and non-diarrheic pediatric patients in Iran. Gut Pathog 5, 39 (2013).

[19] Moses, A.E., Udo, S.M., Bassey, B.E. & Egwu, G.O., (2010). Detection of *Escherichia coli* O157 and non O157 serogroups in cattle faces, dairy products and water samples in North east Nigeria. Journal of Medical Laboratory Science, Vol 19, No1

[20] Mühlen, S. and Dersch, P. (2020) Treatment Strategies for Infections With Shiga Toxin-Producing *Escherichia coli*. Front. Cell. Infect. Microbiol. 10:169.

[21] Muhlen, S., Ramming, I., Pils, M. C., Koeppel, M., Glaser, J., Leong, J., et al. (2020). Identification of antibiotics that diminish disease in a murine model of enterohemorrhagic *Escherichia coli* infection. Antimicrob. Agents Chemother. 64.

[22] Nassar, F. J., Rahal, E. A., Sabra, A., and Matar, G. M. (2013). Effects of subinhibitory concentrations of antimicrobial agents on *Escherichia coli* O157:H7 shiga toxin release and role of the SOS response. Foodborne Pathog. Dis. 10, 805–812.

[23] Natália, M.N., Rachel, Z.S., Dalila, A.R., John, M.F. & Oswaldo, D.R.J., (2014). Occurrence of Escherichia coli O157:H7 and O26 sorbitol negative in a cattle slaughterhouse and susceptibility to antimicrobials. Arq. Institute of Biology, Sao Paulo, v.81, n.3, p. 209-217, 2014.

[24] Pan, Y., Bin, H., Bai, X., Yang, X., Cao, L., Liu, Q., Sun, H., Li, H., Zhang, J., Jin, D. and Xiong, Y. (2021). Antimicrobial Resistance of Non-O157 Shiga Toxin-Producing *Escherichia coli* Isolated from Humans and Domestic Animals. Antibiotics 2021, *10*(1), 74

[25] Peron, E., Zaharia, A,, Zota, L.C., Severi, E., Mårdh, O., Usein, C. , *et al.* (2016). Early findings in outbreak of haemolytic uraemic syndrome among young children caused by Shiga toxin-producing *Escherichia coli*, Romania, January to February 2016. Europiam Surveillance. 2016;21(11):30170.

[26] Reiji, H., Masakado, M., Yoshio, M., Yasumoto, S., Makoto, S. & Yutaka, M., (2002). Characterization of Shiga Toxin-Producing *Escherichia coli* O26 Strains and Establishment of Selective Isolation Media for These Strains. Journal of Clinical Microbiology, Mar. 2002, p. 922–925 Vol. 40, No. 3.

[27] Reza, R., Farhad, S.D., Mohammad, H., Sakhaei, S. & Ebrahim, R., (2018). Prevalence, identification of virulence factors, O-serogroups and antibiotic resistance properties of Shiga-toxin producing *Escherichia coli* strains isolated from raw milk and traditional dairy products. Antimicrobial Resistance and Infection Control (2018) 7:53.

[28] Rosales, A., Hofer, J. & Zimmerhackl, L.B., (2012). Need for long-term followup in enterohemorrhagic *Escherichia coli*-associated hemolytic uremic syndrome due to late-emerging sequelae. Clinical Infectious Diseases 2012; 54:1413–21.

[29] Russo, T.A., (2001). Harrison's principles of internal medicine, 15th ed. McGraw-Hill Professional, New York, N.Y.

[30] Ruusunen, M., Salonen, M., Pulkkinen, H., Huuskonen, M., Hellström, S., Revez, J., Hänninen, ML., Fredriksson-Ahomaa, M. & Lindström, M., (2013). Pathogenic bacteria in Finnish bulk tank milk. Foodborne Pathogical Diseases 10:99 –106.

[31] Salma, A.L., Jeremy, B. & Robert, B., (2017). Survival capabilities of Escherichia coli O26 isolated from cattle and clinical sources in Australia to disinfectants, acids and antimicrobials. BMC Microbiology (2017) 17:47.

[32] Saridakis, H.O., (1994). Non production of Shiga-like toxins by *Escherichia coli* serogroup O26. Review Microbiology 25, 154-155.

[33] Sasaki Y.,, Usui, M., Murakami, M., Haruna M., Kojima, A., Asai, T., and Yamada, Y. (2012). Antimicrobial Resistance in Shiga Toxin-Producing *Escherichia coli* O157 and O26 Isolates from Beef Cattle. Japan. Journal Infection Disease., 65, 117-121, 2012

[34] Scotland, S.M., Willshaw, G.A., Smith, H.R. & Rowe, B., (1990). Properties of strains of *Escherichia coli* O26:H11 in relation to their enteropathogenic or enterohemorrhagic classi¢cation. Journal of Infectious Diseases. 162, 1069-1074.

[35] Tarr, P.I., Gordon, C.A. & Chandler, W.L., (2005). Shiga toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. Lancet 2005; 365:1073–86.

[36] Trabulsi, L.R., Campos, L.C., Wittham, T.S., Goncalves, T.A.T., Rodrigues, J. & Goncalves., A.G., (1996). Traditional and non-traditional enteropathogenic *Escherichia coli* serogroups. Review Microbiology 27, 1-6.

[37] Yoshikazu, I, Soichiro, K., Jimena, A., Katsuaki, S., Masanobu, O., Naotaka, H., Kazumichi, T. & Keizo, Y., (2005). Extended-Spectrum-Lactamase-Producing Shiga Toxin Gene (*stx*1)-Positive *Escherichia coli* O26:H11: a New Concern. Journal of Clinical Microbiology, Mar. 2005, p. 1072–1075.