Occurrence and Antibiogram of Escherichia Coli O157:H7 in Locally Fermented Milk (Nono) Sold Under Market Conditions in Nasarawa State, Nigeria

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Abstract: ***Escherichia coli O157:H7 is a newly emerging pathogen frequently associated with the consumption of food of bovine origin. Severe and life threatening human diseases caused by E. coli O157:H7 strains have been reported throughout the world. This present study evaluated the occurrence of E. coli O157:H7 in locally-fermented milk (nono) sold under market conditions in Nasarawa State, Nigeria and the patterns of their antibiotic susceptibility. A total of 420 nono samples were purchased across Nasarawa State. The samples were bacteriologically analyzed in the laboratory for the presence of E. coli O157:H7 by means of cultural techniques (enrichment on modified tryptone soy broth and selective plating on Cefixime-Tellurite Sorbitol Mac-Conkey Agar), biochemical (Microbact 12E) and serological assays. Oxoid diagnostic kit; latex (R30959601) was used to confirm E. coli O157:H7 respectively. Confirmed isolates were further subjected to antimicrobial susceptibility test using the Agar disc diffusion technique. The results of the study showed that out of 420 nono samples examined, 19 (4.5%) were contaminated with E. coli O157:H7. Among samples examined, the highest occurrence rate (5.7%) was recorded in samples obtained from Akwanga, Wamba and Doma Local Government Areas while Lafia and Keffi had the least occurrence rate (2.9%). With respect to the senatorial zones, Nasarawa North had the highest occurrence rate of 5.7% while the Southern zone had the least (3.6%). There was no significant difference (P>0.05) in the occurrence of E. coli O157:H7 isolated from nono samples with respect to the various Local Government Areas. Antibiotics susceptibility profile showed that all the isolates were resistant to multiple antibiotics, except ciprofloxacin and gentamicin, resulting in nine different resistance patterns. All the nineteen (100%) of the isolates were resistant to penicillin and tetracycline, 18 (94.7%) to erythromycin, 16 (84.2%) to amoxicillin, oxacillin and sulphamethoxazole/trimethoprim, 13 (68.4%) to chloramphenicol and 8 (42.1%) to streptomycin. 15 (78.9%) and 17 (89.5%) of the isolates were sensitive to ciprofloxacin and gentamicin. The predominant antimicrobial resistance pattern was penicillin-tetracycline-chloramphenicol-amoxycilin-erythromycin-oxacillin-sulphamethoxazole/ trimethoprim with the occurrence rate of 36.8% from samples obtained from Wamba, Doma, Kokona and Keffi. Nono consumption has potential health risks to consumers in Nasarawa State, hence proper hygiene in the process-line and marketing of nono is recommended. The multiple antimicrobial resistance exhibited by E. coli O157:H7 strains in this study is an indication of possible antibiotic abuse.***

Keywords: E. coli O157:H7, emerging, locally fermented milk, Antibiotics, resistance.

1. Introduction

One of the most significant food-borne pathogen that has gained increased attention in recent years is the E. coli O157:H7. E. coli O157:H7 was first recognized as food pathogen in 1982 [1]. Since then, it has become a pathogen of major concern in both food and dairy industries, and to the public, because of its ability to cause severe illness, in particular, haemorrhagic colitis, haemolytic uremic syndrome and thrombotic thrombocytopenic purpura [2]. Domestic and wild animals are the sources of E. coli O157:H7, but ruminants are regarded as the natural reservoir. Sporadic cases and outbreaks of human diseases caused by E. coli O157:H7 have been linked to ground beef, raw milk, meat and dairy products, vegetables, unpasteurized fruit juices and water [3]. Infection can also be acquired by direct contact with animals and by person-to-person spread [4]. In different countries, people have suffered from many infections caused by E. coli O157:H7. This bacterium has been found in healthy bovine faeces. Therefore, milk and milk products produced from such animal milk may pose a risk of infection if the milk is not adequately handled [5].

E. coli O157:H7 has become important pathogen with worldwide distribution [6]. Food borne infection with E. coli O157:H7 continues to be a significant public health problem in Nigeria. A great proportion of the milk produced in tropical countries is converted into indigenous products like ghee or some kind of fermented or concentrated products that are kept without artificial cooling [7]. Various bacteriological analyses of locally-fermented milk (nono) revealed various significant prevalence rates of the occurrence of E. coli, especially serotype O157:H7. Escherichia coli frequently contaminate milk and milk products and it is a good indicator of faecal or environmental contamination [8], [9]. E. coli may gain entry into raw milk directly from dairy cows experiencing sub-clinical or clinical mastitis [10]; from the farm environment particularly the water source and utensils used for the storage of milk on farm or during transportation [11], [12].

Although antibiotics are not recommended for treatment of E. coli O157:H7 infections in humans, there is evidence that bacterial isolates are resistant to some antibiotics [13]. Because some antibiotics may cause bacterial lysis and liberate the free Shiga toxins in the intestinal tract [14], [15], the antimicrobial treatment is contraindicated for human E. coli O157:H7 infections. However, such treatments may be recommended for cystitis and pyelonephritis other than haemorrhagic colitis all caused by E. coli O157:H7 [16]. For those limitations of using antimicrobial agents in E. coli O157:H7 cases, the generally accepted belief is that the E. coli O157:H7 may still be susceptible to most antimicrobials. In addition to their epidemiological importance, the studies of antimicrobials susceptibility of E. coli O157:H7 may have more therapeutic significance as recent studies have indicated a possible role of early administration of antimicrobials in preventing the progression of haemolytic uremic syndrome and haemorrhagic colitis both caused by E. coli O157:H7 [17].

This study determined the prevalence of E. coli O157:H7 in locally-fermented milk (nono) sold under market conditions in Nasarawa State, Nigeria and their antibiotic resistance patterns.

2. Materials and Methods

2.1 Study Area

Nasarawa State falls within the Guinea Savannah zone and covers an area of about 10,470 square kilometres and between longitude 7^0 51'58'' East and latitude 8^0 21'58'' North in central Nigeria. It is characterized by two main seasons: a rainy season (April to September), and a dry season (October to March). It is bounded in the North by Kaduna State, in the West by the Abuja Federal Capital Territory, in the South by Kogi and Benue States and in the East by Taraba and Plateau States. Nasarawa State has a projected human population of 2,040,097, having agriculture as the mainstay of its economy with the production of varieties of cash crops throughout the year [47].

2.2 Sample Collection and Handling

Four hundred and twenty (420) nono samples were obtained across Nasarawa State, Nigeria between April to November, 2012 for this study. Seventy (70) nono samples were randomly purchased from nono hawkers in each of the Local Government Area selected for this study viz: Akwanga and Wamba (Nasarawa North), Lafia and Doma (Nasarawa South) and Keffi and Kokona (Nasarawa West) respectively. Convenient sampling method was used for sampling. The selection of these areas was based on cattle population and nono hawkers. Each sample (25ml) was collected into a sterile corked plastic tube and then labelled appropriately. All samples were placed in separate sterile plastic bags to prevent spilling and cross contamination. Samples were stored in a cooler with ice packs and then transported to the Bacterial Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University Zaria, for Laboratory analysis within 4 to 5hours.

2.3 Isolation of E. coli O157:H7

The steps for the isolation of E. coli O157:H7 were conducted according to the isolation procedure of ISO [45]. These steps included: Enrichment, Selective plating, biochemical characterization and serological confirmation by latex agglutination.

2.4 Enrichment

25ml of the nono sample was directly added to 225ml modified Tryptone soy broth supplemented by novobiocin (mTSB+N). The inoculated broths were incubated at 37^oC for 24hours.

2.5 Selective Plating and Identification of E. coli O157:H7 Colonies

A loopful of the enriched sample (inoculated broth) was streaked onto Telluritte-Cefixime Sorbitol MacConkey agar (CT-SMAC agar) plate and incubated at 37^oC for 24 hours. A typical E. coli O157:H7 appeared as a non-sorbitol fermenter colony (NSFC) which is characterized as having a slightly transparent, almost colourless with a weak pale brownish appearance. About 4 to 5 colonies from the CT-SMAC agar plates were picked and sub-cultured onto non-selective medium, such as nutrient agar slants at 37^oC for 24hours, and refrigerated for further biochemical and serological analysis. Also, NSFC were examined for the presence of gram negative rods by gram staining.

2.6 Biochemical Test

The strains were characterized biochemically using Microbact 12E ($MB1130A^+$, Oxoid) according to the manufacturer's instruction. Identification was done following a series of 12 biochemical tests.

2.7 Serological Test

E. coli positive colonies were serologically confirmed by using E. coli O157:H7 latex agglutinations assay (R30959601, Oxoid), containing latex particles coated with antibodies specific for E. coli O157 and E. coli H7 antigen. Identification of E. coli O157:H7 was carried out following the manufacturer's instruction, hence colonies that agglutinated were considered to be E. coli O157:H7.

2.8 Antimicrobial Susceptibility Test

The isolates were screened for antimicrobial susceptibility, using the agar disk diffusion method by Kirby-Bauer [46]. The following antibiotics (Oxoid) were used: Penicillin (10 units), Gentimicin (10µg), Ciprofloxacin (5µg), Streptomycin (10µg), Amoxycillin (25µg), Tetracycline (30µg), Chloramphenicol (30µg), Oxacillin (5µg), Erythromycin (5µg) and Sulphamethoxazole/Trimethoprim (25µg).

The isolates were uniformly streaked on Muller-Hinton agar plate and the antibiotic impregnated discs were applied onto the inoculated plates using sterile forceps. The plates were then incubated at 37^{o}C for 24hrs, after which clear zones of inhibition for each antibiotic were measured using transparent ruler. The results were interpreted using the Clinical and Laboratory Standards Institute (CLSI) criteria [44].

3. Results

3.1 Prevalence of E. coli O157:H7

Table 1 summarizes the results of the occurrence of E. coli O157:H7 in locally fermented milk (nono) samples obtained from various Local Government Areas in Nasarawa State. Out of the 420 nono samples examined, 19 (4.5%) were found to be contaminated with E. coli O157:H7. The results also indicated the percentages of the occurrence of the pathogen across the selected Local Government Areas in Nasarawa States viz: Akwanga 4 (5.7%), Wamba 4 (5.7%), Lafia 2 (2.9%), Doma 4 (5.7%), Keffi 2 (2.9%) and Kokona 3 (4.3%) respectively.

Table 2 indicates the prevalence rates of E. coli O157:H7 across the senatorial zones of Nasarawa State thus: Nasarawa North (comprising of Akwanga and Wamba) 8 (5.7%), Nasarawa West (comprising of Keffi and Kokona) 5 (3.6%) and Nasarawa South (comprising of Lafia and Doma) 6 (4.3%).

Table 3 shows the occurrence of E. coli O157:H7 with respect to the market sites. Nono sold in Market areas harbours 15 (5.0%) of E. coli O157:H7 while those sold in Streets and Schools were contaminated with 3 (3.2%) and 1 (4.2%) of this organism respectively.

Overall, E. coli O157:H7 occurred equally in some of the Local Government Areas and varied in some, with percentage occurrence of the organism ranging from 2.9% to 5.7% (table 1).

3.2 Antimicrobial Susceptibility of E. coli O157:H7

Table 4 shows the antimicrobial susceptibility patterns of the 19 E. coli O157:H7, isolated from locally fermented milk (nono) samples, using 10 antibiotics. 17 (89.5%) of the isolates were sensitive to gentamicin, 15 (78.9%) were sensitive to ciprofloxacin, 8 (42.1%) were sensitive to streptomycin, 3 (15.8%) were sensitive to oxacillin and sulphamethoxazole / trimethoprim, 1 (5.3%) was sensitive to chloramphenicol and amoxicillin and none was sensitive to penicillin, tetracycline and erythromycin respectively.

Antibiotic susceptibility profile showed that virtually all the isolates were resistant to one or multiple antibiotics. However, 19 (100%) of the 19 E. coli O157:H7 isolates were resistant to penicillin and tetracycline, 18 (94.7%) were resistant to erythromycin, 16 (84.2%) were resistant to amoxicillin, oxacillin and sulphamethoxazole / trimethoprim, 13 (68.4%) were resistant to chloramphenicol, 8 (42.1%) were resistant to streptomycin and none was resistant to ciprofloxacin and gentamicin (Table 4.4). Gentamicin, ciprofloxacin and streptomycin were the most sensitive antibiotics whereas penicillin, tetracycline and erythromycin were the least sensitive. The total percentage resistivity of the antibiotics tested against the E. coli O157:H7 isolates was 51.3% (Table 5). The antimicrobial resistance patterns are shown in Table 6. The most common patterns were penicillin-tetracycline-chloramphenicol-amoxycillin-erythromycin-oxacillin- sulphamethoxazole / trimethoprim 7 (36.8%), penicillin – streptomycin – tetracycline – chloramphenicol - amoxycillin-erythromycin-oxacillin- sulphamethoxazole / trimethoprim 3 (10.5%), penicillin-strepromycin-tetracycline-amoxycillin-erythromycin-oxacillin sulphamethoxazole/trimethoprim 2 (10.5%) and penicillin-tetracycline-amoxycillin-erythromycin-oxacillin-sulphamethoxazole/trimethoprim 2 (10.5%). Penicillin and tetracycline resistance were most common among the various patterns observed. The highest levels of multidrug resistance observed were in isolates from Wamba, Doma, Kokona and Keffi Local Government Areas respectively. The relationship between the patterns of antibiotic resistance and number of isolates showing such pattern is presented in Table 7.

Table 1: Prevalence of E. coli O157: H7 in Locally-Fermented Milk (Nono) Sold in Nasarawa State, Nigeria

LGA	*No. (%) of Samples*	*No. (%) Positives*
Akwanga	70 (16.7)	4 (5.7)
Wamba	70 (16.7)	4 (5.7)
Lafia	70 (16.7)	2 (2.9)
Doma	70 (16.7)	4 (5.7)
Keffi	70 (16.7)	2 (2.9)
Kokona	70 (16.7)	3 (4.3)
Total	420 (100)	19 (4.5)

Table 2: Prevalence of E. coli O157: H7 in Locally Fermented Milk (Nono) Sold in Nasarawa State Senatorial Zones

Senatorial zone	*No. (%) of sample*	*No. (%) positive*
Nasarawa North	140 (33.4)	8 (5.7)
Nasarawa South	140 (33.4)	6 (4.3)
Nasarawa West	140 (33.4)	5 (3.6)
Total	420 (100)	19 (4.5)

Table 3: Occurrence of E. coli O157: H7 from Locally Fermented Milk (Nono) with Respect to Market Sites

Site	*No. Examined*	*No. (%) positive*
Market	302	15(5.0)
Street	93	3(3.2)
School	25	1(4.2)
Total	420	19 (4.5)

Table 4: Antimicrobial Susceptibility Pattern of E .coli O157:H7 Isolates from Locally Fermented Milk (Nono) Sold in Nasarawa State.

		Susceptibility		
		R	*I*	*S*
Antibiotic	*Concentration (µg)*	*No. (%)*	*No. (%)*	*No. (%)*
Penicillin	10	19 (100.0)	0 (0.0)	0 (0.0)
Ciprofloxacin	5	0 (0.0)	4 (21.1)	15 (78.9)
Gentamicin	10	0 (0.0)	2 (10.5)	17 (89.5)
Streptomycin	10	8 (42.1)	3 (15.8)	8 (42.1)
Tetracycline	30	19 (100.0)	0 (0.0)	0 (0.0)
Chloramphenicol	30	13 (68.4)	5 (26.3)	1 (5.3)
Amoxicillin	25	16 (84.2)	2 (10.5)	1 (5.3)
Erythromycin	5	18 (94.7)	1 (5.3)	0 (0.0)
Oxacillin	5	16 (84.2)	0 (0.0)	3 (15.8)
Sulphamethoxazole / Trimethoprim	25	16 (84.2)	0 (0.0)	3 (15.8)

Table 5: Percentage Resistivity of the Tested Antibiotics against E. coli O157:H7 Isolates from Nasarawa State.

LGA	*No. of Isolates*	*No. (%) of Antibiotic Resistant*
Akwanga	4	26(65)
Wamba	4	24 (60)
Lafia	2	13 (32.5)
Doma	4	29 (72.5)
Keffi	2	12 (30)
Kokona	3	19 (47.5)
Total	19	123 (51.3)

Table 6: Antimicrobial resistance Patterns of 19 Isolates of E. coli O157:H7 from Locally Fermented Milk (nono) Sold in Nasarawa State.

No. of Antibiotics	*Resistance Pattern*	*No. (%) of Isolates*	*LGA*
4	Pen, Tet, Chl, Ery	1 (5.3)	Wa
4	Pen, Str, Tet, Sul	1 (5.3)	Ak
5	Pen, Str, Tet, Ery, Sul	1 (5.3)	La
6	Pen, Tet, Amo, Ery,Oxa, Sul	2 (10.5)	Ke, Ko
6	Pen, Tet,Chl, Amo, Ery,Oxa	1 (5.3)	Wa
7	Pen, Tet, Chl, Amo, Ery, Oxa, Sul	7 (36.8)	Wm, Do, ko, ko
7	Pen, Str, Tet, Amo, Ery, Oxa, Sul	2 (10.5)	Do, Ak
7	Pen, Str, Tet, Chl, Amo, Ery, Oxy	1 (5.3)	Ak
8	Pen, Str, Tet, Chl, Amo, Ery,Oxa, Sul	3 (15.8)	Do, Ak, La

Table 7: The Relationship between the Patterns of Antibiotic Resistance and Number of Isolates

S/N	*No. of Antibiotic*	*No. (%) of Isolates*
1	4	1 (5.3)
2	4	1 (5.3)
3	5	1 (5.3)
4	6	2 (10.5)
5	6	1 (5.3)
6	7	7 (36.8)
7	7	2 (10.5)
8	7	1 (5.3)
9	8	3 (15.8)

4. Discussion

In some developed countries, E. coli O157:H7 has been detected in raw milk and milk products [18]. Likewise the result obtained from this research revealed that this organism was present in locally fermented milk (nono) marketed in Nasarawa State, Nigeria. Apart from detecting E. coli O157:H7 from bovine milk, it has also been documented that bovine milk is a vehicle for E. coli O157:H7 infection [18]-[23].

The percentage occurrence of E. coli O157:H7 in locally fermented milk (nono) in Nasarawa State recorded in this study is 4.5%. This is higher than the data reported in Plateau State, Nigeria, where 5 (0.71%) out of 350 nono samples and 21 (3.00%) from 350 raw milk samples were detected [24]. The occurrence of this organism in this study is also higher than the data reported in raw milk from the United States of America in which out of the 1, 021 bovine milk samples examined, 20 (2.0%) were positive for E. coli O157:H7 [18]. Lower occurrence rate of E. coli O157:H7 (O.5%) in traditional dairy products was recorded from a study in Iran [25] as compared with the result obtained from this study.

The significance of the isolation of E. coli O157:H7 from this local milk product (nono) is of general public health concern because many people in Nigeria, mostly in the northern States consume this product. Thus, the product may be one of the major vehicles for E. coli O157:H7 transmission from cattle to man.

There is no significant difference ($P>0.05$) in the occurrence of E. coli O157:H7 isolated from nono samples with respect to the various Local Government Areas, indicating that the milk produced and marketed in these areas may have similar microbial quality. This may be due to the fact that similar handling procedures are employed during milking process and during the production of fermented milk (nono) in the various areas.

The relatively higher occurrence of E. coli O157:H7 in locally fermented milk (nono) in Nasarawa State, Nigeria may be attributed to lack of effective sanitary precautions and less careful handling procedures during milking process and nono production. The use of traditional milking methods also expose milk to pathogenic bacteria found in cow udders and probably on the hands of the milkers who may have come in contact with faeces of the cows. The unhygienic environmental conditions where nono is marketed may also contribute to its contamination. The use of more sensitive assay such as enrichment broth and selective media for the isolation of E. coli O157:H7 in this study might have helped in obtaining a higher occurrence of the organism. In a study in USA by [26], E. coli O157:H7 was not isolated from any of the milk samples examined. These authors suggested that the absence of the organism in milk was as a result of the use of assay which was insensitive to E. coli O157:H7.

The antimicrobial sensitivity tests showed a high level of resistance to most of the antibiotics used. The

development of antimicrobial resistance by the bacteria to these drugs poses a major challenge in both human and animal medicine because these drugs are commonly used in the treatment of human patients and in veterinary practice. Penicillin and tetracycline resistance were the highest. All the isolates (100%) tested were resistant to both antibiotics. This is in agreement with the finding of [27], which recorded a high level of tetracycline resistance of 91.4% among isolates of E. coli O157:H7. Al Haj [28] also observed high resistance to tetracycline (81.4 %); [29] obtained also high resistance to penicillin (72%) and to Tetracycline (57.9%); O'Brien [47], also reported high resistance to tetracycline (72%). The high level of resistance of tetracycline obtained in this study may be as a result of it being the most commonly available antibiotic used as growth promoter and routine chemoprophylaxis among livestock in Nigeria [27]. This is worrisome considering that tetracycline is a first line drug in Nigeria, and as in most developing countries, people with gastrointestinal infections readily purchase it across the counter for self-medication [30]. Penicillin resistance as obtained from this study may be as a result of the frequent usage of this antibiotic in treating diseases in cattle [31]. According to Hart and Kariuki and Okeke penicillin and tetracycline are known to be extensively used in developing countries [32]-[33].

High rate of resistance to sulphamethoxazole / trimethoprim (84.2%) is in agreement with the previous work by Shroeder, who reported that among 189 E. coli O157:H7 isolates recovered from various sources between 1985 and 2000, 19 (10%) were resistant to this antibiotic [34]. This antimicrobial is commonly used to treat respiratory infections, diarrhoea, mastitis, and other infections in beef and dairy cattle. Resistance was found to be relatively low in streptomycin. This probably may be because of less exposure to the antibiotic due to the discourage use of the antibiotic and the fact that it is usually administered intravenously thereby restricting indiscriminate use [35]. This shows that streptomycin can be used as an antibiotic of choice against E. coli O157:H7 infections, except for its serious side effect [36].

The high prevalence of resistance of E. coli O157:H7 isolates to erythromycin, oxacillin, amoxicillin and chloramphenicol is of importance from the view point of medical and veterinary practice in Nigeria. This could be a reflection of use and misuse of these antibiotics in the society. This finding is not surprising because outside the hospital environment the general population have easy access to various antibiotics at any drug store without any prescription from a medical practitioner.

In this study, all the E. coli O157:H7 isolates tested showed multidrug resistance to the antibiotics tested at various percentages. This result is in agreement with the findings by other researchers, who reported multidrug resistance among E. coli O157:H7 isolates [37], [34]. Various isolates were resistant to 4, 5, 6, 7 and 8 of the antibiotics tested. Isolates from Doma, Akwanga and Lafia showed higher frequencies of multidrug resistance. Multiple antimicrobial resistances in E. coli O157:H7 isolates may partly result from the spread of genetic elements including plasmids, transposons, and integrons [38] that may confer resistance to numerous antimicrobials. According to Aarestrup and Levin et al., multiple resistances capable of regional dissemination can emerge as a result of antimicrobial selection pressure in either livestock or humans [39]-[40]. Evidence has been found which indicates that resistant strains of pathogens can be transmitted to humans through food [41]-[42].

Results from this study indicate that ciprofloxacin (floroquinolone) and gentamicin (aminoglycoside) are the drugs of choice for E. coli O157:H7, since none of the isolates was resistant to them. This shows the effectiveness of the floroquinolones and aminoglycosides, and is in agreement with the finding of Scheld, suggesting the use of this class of antibiotics [43].

The public health significance of these findings is that antimicrobial resistant E. coli O157:H7 from nono (or dairy animals) may colonize the human population via the food chain, consumption of nono, contact through occupational exposure, or waste runoff from nono production facilities to the neighbourhood. Indiscriminate use of antimicrobials among livestock producers and marketers in Nigeria could also be responsible for the resistance pattern obtained in this study.

5. Conclusion

The detection of E. coli O157:H7 in locally-fermented milk (nono) sold in Nasarawa State, suggests that nono consumption has potential health risks to consumers in Nasarawa State, Nigeria. The antibiogram of E. coli O157:H7 isolates showed a high prevalence of resistance to most of the antibiotics used. The data suggest that selection pressure imposed by the use of these antibiotics whether therapeutically in human and veterinary medicine or as prophylaxis in the animal production, is a key driving force in the selection of antimicrobial resistance in E. coli O157:H7. There is a need to legislate and enforce laws to limit the prescription and dispensing of antibiotics and other drugs to only qualified professionals. Education of the public on the dangers of indiscriminate purchase and use of drugs is also imperative.

References

[1] W.L. Riley, R.S. Remis, S.D. Helgerson, H.B. McGee, J.D. Wells, B.R. Davis, M.L. Cohen, "Haemorrhagic Colitis Associated with a Rare Escherichia coli Serotype," New England Journal of Medicine, 308(12), pp 681-685, 1983

[2] M.U. Abdul-Raouf, R.L. Beuchat, S.M. Ammar, "Survival and Growth of E. coli O157:H7 in Ground, Roasted Beef as Affected by pH, Acidulants and Temperature," Journal of Applied Environmental Microbiology, 59980, pp 2364-2368, 1993

[3] P.A. Chapman, C.A. Siddons, A.T. Cerdan-Malo, A.M. Harkin, "A 1-Year Study of E. coli O157:H7 in Cattle, Sheep, Pigs and Poultry," Journal for Epidemiology and Infections. 119, pp245-250, 1997

[4] S. Cho, F. Diez-Gonzales, C.P. Fossler, S.J. Wells, C.W. Hedberg, J.B. Kaneene, "Prevalence of Shiga Toxin-Encording Bacteria and Shiga-Producing E.

coli Isolates from Dairy Farms and Country Fair," Journal of Veterinary Microbiology, 118, pp 289-298, 2006
[5] M. Arici, S. Kurultay, T. Gumus, "Incidence of Escherichia coli 0157 in Raw Milk and White Pickled Cheese Manufactured from Raw Milk in Turkey," Journal for Food Control," 15(6),pp 453-456, 2004
[6] J. Schlundt, "New Directions in Foodborne Disease Prevention," International Journal of Food Microbiology, 78, pp 3-17, 2002.
[7] E.C. Okolocha, P.T.A. Egwu, J.U. Umoh, I.L.I. Luga, "Hazards and Critical Control Points of Yoghurt Produced in a Research Institute Farm in Zaria, Nigeria," Nigeria Veterinary Journal, 27(3), pp 15-24, 2008
[8] A.H. Soomro, M.A. Arain, M. Khaskheli, B. Bhutto, "Isolation of Escherichia coli from Raw Milk Products in Relation to Public Health Sold Under Market Condition at Tandojam; Pakistan," Journal for Nutrition, 1(3), pp.151-152, 2002
[9] N. Benkerroum, Y. Bouhal, E.I. Attar, A. Marhaben, "Occurrence of Shiga Toxin-producing Escherichia coli 0157:H7 in Selected Diary and Meat Products Marketed in the City of Rabat, Morocco," Journal Food Protection, 67(6), pp.1234-1237, 2004
[10] D. Rodojcic-Prodaova, T. Necer, "Most Common Agents of Subclinical Mastitis in Cows on Private and Communal Farms in the Republic of Macedonia," British Journal of Diary Science, 45, pp. 745-749, 1991.
[11] R.J. Eberhart, "Coliforms Mastitis," Journal of American Veterinary Association, 170, pp.1160-1163, 1977.
[12] B. Freedman, "Milk Quality in Sanitarials Handbook: Theory and Administrative Practice for Environmental Health," 4th Edition., Peerless Publishing, New Oreleans, USA, 1977.
[13] I.E. Aibinu, R.F. Peters, K.O. Amisu, S.A. Adesida, M.O. Ojo, O. Tolu, "Multidrug Resistance in E. coli O157 Strains and the Public Health Implication," Journal for Animal Science, 3(3), 22-33, 2007.
[14] H. Karch, J. Heesemann, R. Laufs, "Phage-Associated Cytotoxin Production by and Enteroadhesiveness of Enteropathogenic Escherichia coli Isolated from Infants with Diarrhea in West Germany," Journal of Infectious Diseases, 155, pp. 707-71 5, 1987.
[15] C.S. Wong, S. Jelacic, R.L. Habeeb, S.L. Watkins, P.I. Tarr, "The Risk of the Hemolytic-Uremic Syndrome after Antibiotic Treatment of Escherichia coli O157:H7 Infections," New England Journal of Medicine, 342:1930-6, 2000.
[16] P.M. Griffin, R.V. Tauxe, "The Epidemiology of Infections Caused by Escherichia coli O157:H7, Other Enterohemorrhagic E. coli, and the Associated Haemolytic Uremic Syndrome," Epidemiologic Reviews, 13, 60-98, 1991.
[17] K. Molbak, P.S. Mead, P.M. Griffin, "Antimicrobial Therapy in Patients with Escherichia coli O157:H7 Infection," Journal of American Medical Association, 288, pp.1014-6, 2002.
[18] G.L. Armstrong, J. Hollingsworth, J.G.J. Morris, "Emerging Food borne Pathogens: Escherichia coli O157:H7 as a Model of Entry of a New Pathogen into the Food Supply of the Developed World,' Epidemiologic Reviews, 18, pp. 29–51, 1996.
[19] A.A. Borczyk, M.A. Karmali, H. Lior, L.M. Duncan, "Bovine Reservoir for Verotoxin-Producing Escherichia coli O157:H7," Lancet, 1, pp. 98, 1987.
[20] J.G. Wells, L.D. Shipman, K.D. Green, "Isolation of Escherichia coli from Diary Cattle," Journal of Clinical Microbiology, 12, p. 23-30, 1999.
[21] G.M. Morgan, C. Newman, S.R. Palmer, J.B. Allen, W. Shepherd, A.M. Rampling, R.E. Warren, R.J.F. Gross, S.M. Scotland, H.R. Smith, "First Recognized Community Outbreak of Haemorrhagic Colitis due to Verotoxin-Producing Escherichia coli O157:H7 in the UK," Journal of Epidemiology and Infection, 101, pp.83–91, 1988.
[22] S.M. Ostroff, J.M. Kobayashi, J.H. Lewis, "Infections with Escherichia coli O157:H7 in Washington State--the First Year of Statewide Disease Surveillance," Journal of American Medical Association, 262, pp.355-359, 1989.
[23] J.E. Coia, "Clinical, Microbiological and Epidemiological Aspects of Escherichia coli O157 Infection,"FEMS Immunology and Medical Microbiology Journal, 20, pp.1–9, 1999.
[24] U.J. Itelina, S.E. Agina, "Occurrence of E. Coli o157:H7 in Locally Fermented Milk (Nono) in Plateau State, Nigeria," Global Journal of Agricultural Sciences. 9 (2), pp. 23-26, 2010.
[25] E. Rahimi, H. Momtaz, M. Mohammad, M. Alimoradi, M. Momeni, M. Riahi, "Isolation and Genomic Characterization of Escherichia coli O157: NM and Escherichia coli O157:H7 in Minced Meat and Some Traditional Dairy Products in Iran," African Journal of Biotechnology, 11 (9), 2328-2332, 2012.
[26] R.C. Clark, S.A. McEwen, V.P. Cannon, "Isolation of Verocytotoxin- Producing Escherichia coli from Milk in South-Western Ontario" Epidemiologic Infections, 102, pp.253-260, 1989.
[27] I.O. Olatoye, "The Incidence and Antibiotics Susceptibility of Escherichia coli O157:H7 from Beef in Ibadan Municipal, Nigeria, "African Journal of Biotechnology, 9(8), pp. 1196-1199, 2010.
[28] N. Al Haj, N.S. Mariana, A.R. Raha, Z. Ishak, "Prevalence of Antibiotic Resistance among Escherichia coli from Different Sources. Malaysia Research," Journal of Pharmacology. 1(2), pp. 44-49, 22007.
[29] A. Shitandi, A. Sternesjö, "Detection of Antimicrobial Residues in Kenyan Milk," Journal of Food Safety, 21, pp. 205-215, 2001.
[30] V.N. Chigor, J.V. Umoh, I.S. Smith, O.E. Igbinosa, I.A. Okoh, "Multidrug Resistance and Plasmid Patterns of Escherichia coli O157 and Other E. coli Isolated from Diarrhoeal Stools and Surface Waters from Some Selected Sources in Zaria, Nigeria," International Journal of Environmental Research and Public Health, 7, pp.3831-3841, 2010.
[31] C.M. Byrne, I. Erol, J.E. Call, C.W. Kaspar, D.R. Buege, C.J. Hiemke, P.J. Fedorka-Cray, A.K. Benson, F.M. Wallace, J.B. Luchansky, "Characterization of Escherichia coli O157:H7 from Downer and Healthy Dairy Cattle in the Upper Midwest Region of the

United States," Journal of Applied and Environmental Microbiology. 69(8), pp.4683-4688, 2002.
[32] A. Hart, K.S. Ariuki, "Antimicrobial Resistance in Developing Countries," British Medical Journal, 317, pp. 647-650, 1998.
[33] N. Okeke, A. Lamikana, R. Edelman, "Socioeconomic and Behavioral Factors Leading to Acquired Bacterial Resistance in Developing Countries," Emerging Infectious Diseases, 5, pp.18-27, 1995.
[34] C.M. Schroeder, C. Zhao, C. DebRoy, J. Torcolini, S. Zhao, G.D. White, D.D. Wagner, F.P. McDermott, D.R. Walker, J. Meng, "Antimicrobial Resistance of Escherichia coli O157:H7 Isolated from Humans, Cattle, Swine, and Food," Journal of Applied and Environmental Microbiology, 68, pp. 576–581, 2002.
[35] M. Cheesbrough, District Laboratory Practice in Tropical Countries. Part 2. Cambridge University Press, United Kingdom, 2000.
[36] M.L. Prescott, P.J. Harley, A.D. Klein, Microbiology. 6th Edition. Mc Graw Hill publishers, New York, 2005.
[37] H. Kim, M. Samadpour, L. Grimm, C. Clausen, T. Besser, M. Baylor, J. Kobayashi, L.M. Neill, F. Schoenknecht, P. Tarr, "Characteristics of Antibiotic-Resistant Escherichia coli O157:H7 in Washington State, 1984-1991," Journal of Infectious Diseases. 170**,** pp.1606-1609, 1994.
[38] S. Zhao, D.G. White, B. Ge, S. Ayers, S. Friedman, L. English, D. Wagner, S. Gaines, J. Meng, "Identification and Characterization of Integron-Mediated Antibiotic Resistance Among Shiga Toxin-Producing Escherichia coli Isolates," Journal of Applied and Environmental Microbiology, 67**,** pp**.**1558-1564, 2001.
[39] F.M. Aarestrup, "Occurrence of Glycopeptides Resistance among Enterococcus faecium Isolates from Conventional and Ecological Poultry Farms," Journal of Microbial Drug Resistance, 1, pp. 255-257, 1995.
[40] B. Levin, M. Lipsitch, V. Pettot, S. Schrags, R. Anita, L. Simonsen, "The Population Genetics of Antibiotic Resistance," Journal of Clinical and Infectious Diseases. 24, pp. S9-S16, 1997.
[41] J. Oosterom, "Epidemiological Studies and Proposed Preventive Measures in the Fight against Human Salmonellosis" International Journal of Food Microbiology, 12, pp. 41-51, 1991.
[42] G. Khachatourians, "Agricultural Use of Antibiotics and the Evolution and Transfer of Antibiotic Resistant Bacteria," Canadian Medical Association Journal, 159, pp. 1129-1136, 1998.
[43] M.W. Scheld, "Maintaining Floroquinolone Class Efficiency: Review of Influencing Factors," Emerging Infectious Disease Journal, 9(1), pp. 1-9, 2003.
[44] CLSI, "Performance Standards for Antimicrobial Susceptibility Testing; Fifteenth Informational Supplement, M100-S15," Clinical and Laboratory Standards Institute Wayne (CLSI): Chicago, IL, USA, 2005.
[45] ISO, "Isolation and Identification of Enterohaemorrhagic Escherichia coli O157," 1st Edition. International Organization for Standardization, Geneve, Switzerland, 2003.
[46] W.M.M. Kirby, A.W. Bauer, J.C. Sherries, M. Turck, "Antibiotics Susceptibility Testing," American Journal of Pathology. 45, pp. 493-496, 1966.
[47] T.F. O'Brien, "Resistance of Bacteria to Antibacterial Agents: Report of Task Force," Reviews of Infectious Diseases. 9, pp. S244-S260, 1987.
[48] D.P. Sabo, "The Mada of Central Nigeria" 1st Edition, Impact Media Press, Abuja, Nigeria, 2011.

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