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Genetic Characterization of Multiple Antibiotics Resistance Genes of *Escherichia coli* Strain from Cow Milk and Its Products Sold in Abuja, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Antibiotic resistance has remained a global concern. Food pathogens that carry resistance genes will cause public health threat irrespective of their pathogenicity, as this pool of resistance genes are disseminated via food chain. The antibiotic susceptibility of *E. coli* isolates to different antibiotics were investigated and resistance genes were genetically identified by multiplex Polymerase Chain Reaction (PCR). The study aimed to determine the phenotypic antibiotic resistance pattern of the *E. coli* isolates and characterize the antibiotics resistance genes in the *E. coli* isolates from the Cow milk and milk products.

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Results: The study showed that the *E. coli* isolates were Multidrug resistant (MDR) to several antibiotics with resistance pattern of four to ten combinations of antibiotics and the antibiotics resistance occurred mostly in three classes of antibiotics, namely: β-lactams, Amphenicol and Tetracycline. The antibiotics resistance genes expressed in the multidrug resistant *E. coli* isolates were *tetA* 22(78.6%), *dfrA1* 9(32.1%), *blaCITM* and *blaSHV* 3(10.7%), *tetB* and *blaTEM* 1(3.6%), *qnr* 0(0%) and *aac (3)-iv* 0(0%).
Conclusion: The study showed that the cow milk and its products sold on the streets of Abuja are contaminated with resistant *E. coli* that can pose health risk to the consumers. Monitoring and screening for resistant food pathogens in food especially of animal origin is vital to mitigate the prevalence of antibiotics resistance.

Keywords: Antibiotic resistance; Escherichia coli; resistance genes; multidrug resistance.

1. INTRODUCTION

Escherichia coli is recognized as a microorganism highly capable of acquiring and transferring antibiotics resistance genes [1,2]. Commensal *E. coli* strains were initially susceptible to several antibiotics [3], however the uncontrolled and excessive use of antibiotics as growth promoters in food animals to meet economic demands, and the non-specific infection preventions and treatments of food producing animals has led to the development of antibiotics resistance and selective pressure in enteric bacteria, typical of *E. coli* and other Enterobacteriaceae. Antibiotic resistant microbes have become a public health issue [4]. Pathogenic *E. coli* is responsible for several enteric diseases including urinary tract infection. The therapeutic options for the infections differ based on the clinical conditions, on the other hand infections caused by Shiga toxin producing *E. coli*, antibiotics are not usually prescribed for treatment [5].

Antibiotics namely the β-lactams, tetracycline, aminoglycosides, quinolones, macrolides and sulfonamides are used as prophylaxis and growth-promoters in food producing animals [6,7]. These antibiotics could be administered to animals through (intravenous, intramuscular, or subcutaneous injections), orally, topically or through intramammary and intrauterine infusions. The unchecked administration of these antibiotics by farmers in food animals has resulted in abundant residues in the food products such as milk, meat and eggs which has led to increased levels of antimicrobial resistance AMR [8].

Multidrug resistance has been widely observed in *E. coli* isolate more disturbingly is the co-resistance to commonly used antibiotics such as aminoglycosides, fluoroquinolones, tetracycline, phenicols and potentiated sulfonamides [9,10, 11,12,13,14].

The emergence of multidrug resistance in *E. coli* and other infections is a growing public health concern [15,16]. This poses antibiotic management problems [17] and complicates the treatment of infections thus increases both morbidity and mortality rates in humans [18]. The MDR factor accounts for limited therapeutic options [19,20] and discovery of new therapies [21]. Resistance to antibiotics may be intrinsic or acquired through mutations in chromosomal genes and horizontal transfer [22] although the means of acquisition of resistance may vary among bacteria species. Genetic transfer of antibiotics resistance genes among bacteria, conferring resistance characteristics onto pathogenic strains is possible [8] through mobile genetic elements such as integrons, plasmids and insertion sequences [23]. This has led to the rise in the occurrence of (MDR). The increased prevalence of antibiotic resistance genes in *E. coli* isolates from food of animal origin represents a growing concern [24].

The quality of raw cow milk is negatively impacted by indiscriminate use of antibiotics in livestock. These chemical residues in milk may lead to allergic reactions, carcinogenic and teratogenic effects, alterations in the balance of intestinal microbiota, or selection of resistant bacteria [25].

Food matrices like fermented foods, minimally heated and raw foods of animal origin contain high microbial cells and can become potential reservoirs of antibiotics-resistant *E. coli* which are transferrable to humans during consumption [8] and can pose great risks to human health [26]. Although the role of raw milk in the dissemination of antibiotic resistance gene is not clear [27] information on the prevalence,

distribution of antibiotic resistant food pathogens and identification of antibiotic resistance genes in raw cow milk and its products is relevant in food surveillance programs that can facilitate the mitigation of antibiotics resistance burden.

The aim of the study is to evaluate the phenotypic antibiotic susceptibility and characterize resistance genes present in the *E. coli* isolated from the Cow milk and milk products in Abuja.

2. MATERIALS AND METHODS

Six hundred (600) samples of raw cow milk and its products were collected from the six Area Councils of Abuja at various points. They were collected during the dry and wet season of the year between the months of December 2016 – August 2017. Samples were collected in duplicates and put in sterile plastic containers at point of sale. They were labeled and transported in an icebox to the laboratory for immediate analysis.

2.1 Reference Bacteria Strain

Reference bacteria strains of *E coli* ATCC 25922, ATCC 43888 and LMG 21766 were used in the study to serve as positive controls.

2.2 Isolation and Identification of *Escherichia coli*

Ten ml (10 ml) of raw Cow milk samples were aseptically transferred into 90 ml of modified Tryptic Soy broth (mTSBn) (Oxoid Limited, Basingstoke, England) supplemented with 20mg/l novobiocin (Oxoid Limited, Basingstoke, England) homogenized for 2mins in a stomacher (Lab Blender 400, Seward Medical, London, UK) and incubated at 37°C for 18 hours as *E. coli* enrichment step [28]. A loopful of the enriched broth was streaked on the plate of Levine's Eosin Methylene blue agar (L-EMBA), (Oxoid Limited, Basingstoke, England). After overnight incubation at 37°C for 18 hours. Presumptive colonies of *E. coli* (greenish metallic sheen appearance with dark purple centers) were Gram stained, biochemically identified by Microbact™ GNB 24E System Kit (Oxoid Limited, Basingstoke, England).

2.3 Evaluation of the *in vitro* Susceptibility of the *E. coli* Isolates to Antibiotics

Ten antibiotic sensitivity discs (Oxoid Limited, Basingstoke, England) were used namely Cefuroxime Sodium (CXM) (30 µg), Cefpodoxime (CPD) (10 µg), Gentamycin (CN) (30 µg), Chloramphenicol (C) (50 µg), Doxycycline (DO) (30 µg), Imipenem (IPM)(10µg), Amoxycillin (AMC) (30µg), Tetracycline (TE) (30 µg), Pefloxacin (PEF) (5 µg) and Ceftrioxone (CRO) (30 µg). Two to three (2-3) colonies of *E. coli* from appropriate cultures were inoculated into 5 ml tryptone soy broth TSB (Oxoid Limited, Basingstoke, England) and incubated at 37°C until the turbidity approximately 0.5 McFarland's standard. Mueller-Hinton agar (Oxoid Limited, Basingstoke, England) plates were prepared and used according to manufacturers' instructions. Standard microbiological procedure were followed to inoculate the sterile Mueller-Hinton agar (Oxoid Limited, Basingstoke, England) plates, a maximum of 4-5 antibiotics discs were placed on the inoculated plates incubated for 18-24hrs at 37°C thereafter zones of inhibition were measured to the nearest millimetre and diameter zones interpreted [29].

2.4 Molecular Detection

2.4.1 Genomic extraction

The genomic deoxyribonucleic acid DNA was extracted using ZR Genomic DNA™ Miniprep Kit (ZYMO Research Corp. USA) according to the manufacturer's instructions. The extracted DNA samples were stored at -20°C ready for molecular studies (PCR). Multiplex Polymerase Chain Reaction was carried out on twenty-eight *E. coli* isolates that exhibited multidrug resistance [30].

Master Mix: The primer sets for the detection of antibiotics resistance genes in the study were *tetA, tetB qnr blaSHV, blaCITM, blaTEM* and *aac (3)-IV* as shown in Table1.The 5 X Master Mix (NEW ENGLAND BIOLABS®) comprised of 1 X Multiplex PCR Master Mix: 20 mM Tris-HCl (pH 8.9 @ 25°C), 50 mMKCl, 30 mM NH4Cl, 2.5 mM MgCl2, 100 units/ml Taq DNA Polymerase, 0.3 nmM each dNTP 3.2% glycerol, 0.08% IGEPAL® CA-630 and 0.07% Tween® 20. The multiplex PCR contained in the master mix tube was 5µl of genomic DNA extract, 0.3 µM *blaSHV*, 0.3 µM *CITM*, 0.3 µM *TEM*, 0.3 µM *tetA*, 0.3 µM *tetB*, 0.3 µM of *dfrA1*, 0.3 µM *qnr* and 0.3 µM of *aac (3)-IV.* The total volume of reaction mix was made up to 25 µl using (NFH_2O) nuclease free water.

Amplification was performed in Gene Amp 9700 (Applied Biosystems) with initial denaturation at

94°C for 8 min, followed by 32 cycles of denaturation at 95°C for 60s, annealing at 55°C for 70s, extension at 72°C for 2 min and final extension at 72°C for 8 min. The amplicons were ready for electrophoresis or stored in the cold temperature for subsequent analysis.

Table. 1. Primers used for characterization of antibiotic resistance genes by multiplex PCR

Target genes	Sequence	Size of product (bp)	References
*tet*A	(F) GGTTCACTCGAACGACGTCA (R) CTGTCCGACAAGTTGCATGA	577	[31]
*tet*B	(F) CCTCAGCTTCTCAACGCGTG (R) GCACCTTGCTGATGACTCTT	634	[31]
qnr	(F) GGGTATGGATATTATTGATAAAG (R) CTAATCCGGCAGCACTATTTA	670	[32]
dfrA1	(F) GGAGTGCCAAAGGTGAACAGC (R)CGCAGATAAATCACCACAATG	367	[33]
blaSHV	(F) TCGCCTGTGTATTATCTCCC (R)CGCAGATAAATCACCACAATG	768	[34]
blaCITM	(F)TGGCCAGAACTGACAGGCAAA (R) TTTCTCCTGAACGTGGCTGGC	462	[34]
aac (3)-IV	(F) CTTCAGGATGGCAAGTTGGT (R) TCATCTCGTTCTCCGCTCAT	286	[34]
bla TEM	(F) GAGTATTCAACATTTTCGT (R) ACCAATGCTTAATCAGTGA	857	[35]

Table 2. Multidrug resistance pattern of *E. coli* from cow milk samples

No of antibiotics	Combination of antibiotics	MARI (%)
3	CPD, C, CXM	0.3
4	CPD, C CXM, AMC	0.4
4	T, CPD, C, CXM	0.4
5	T, CPD, C, CXM, DO	0.5
5	T, CPD, C, CXM, DO	0.5
5	CPD, C, CXM, DO, AMC	0.5
6	T, CPDC, C, CXM, DO, AMC	0.6
6	T, CPD, C, CXM, CN, DO	0.6
7	T, CPD, C, CXM, PEF DO, AMC	0.7
8	CPD, C, CXM, CRO, PEF, CN, DO, IPM	0.8
10	T, CPD, C, CXM, CRO, PEF, CN, DO, IPM, AMC	1.0

Key: No.= number, MARI = multiple antibiotic resistance index, % = percentage, AMC – Amoxicillin, CPD- Cefpodoxime, CRO- Ceftriaxone, CXM- Cefuroxime Sodium, IMP- Imipenem, PEF- Pefloxacine, CN- Gentamicin, TE- Tetracycline, DO-Doxycycline and C- Chloramphenicol

Table 3. Frequency of occurrence of antibiotics resistance genes in multidrug resistant *E. coli* isolated from cow milk samples

Types of samples	No screened	Antibiotics resistance genes (%)							
		tetA	***tetB***	***qnr***	***dfrA1***	***blaSHV***	***blaCITM***	***blaTEM***	***aac (3)-iv***
Kindrimo	5	5(100)	1(20)	0(0)	0(0)	0(0)	1(20)	0(0)	0(0)
Madara	13	10(76.9)	0(0)	0(0)	5(38.5)	3(23.1)	1(7.7)	1(7.7)	0(0)
Nono	5	3(60)	0(0)	0(0)	3(60)	0(0)	0(0)	0(0)	0(0)
Mashanu	5	4(80)	0(0)	0(0)	1(20)	0(0)	1(20)	0(0)	0(0)
Total	28	22(78.6)	1(3.6)	0(0)	9(32.1)	3(10.7)	3(10.7)	1(3.6)	0(0)

Key: No. = number, % = percentage, tetA and tetB = tetracycline resistance genes, qnr = quinolones resistance gene, dfrA1= trimethroprim resistance gene, blaSHV, blaCITM and blaTEM = extended spectrum beta-lactamase resistance genes

2.4.2 Electrophoresis

Ten microliters (10 µl) of the PCR product was electrophoresed in 1.5% agarose gel containing 5 µl of 10 mg/ml ethidium bromide at 80 volts for 60 min. Hundred (100) bp DNA marker (New England Biolabs®) was used as molecular size marker. The amplicons were viewed under a transilluminator and results documented using gel documentation system (BioRad).

3. RESULTS

Table 2 shows the phenotypic resistance pattern of the multidrug resistant *E. coli*. All the multidrug resistant *E. coli* were resistant to Cefpodoxime (CPD), Chloramphenicol (C) and Cefuroxime Sodium (CXM). Three combination (CPD, C and CXM) had multiple antibiotics resistance index (MARI) of 0.3%. Other combinations of antibiotics were (CPD, C, CXM, AMC) (T, CPD, C, CXM) (T, CPD, C, CXM, DO) (T, CPD, C, CXM, AMC) (CPD, C, CXM, DO, AMC) (T, CPD, C, CXM, DO, AMC) (T, CPD, C, CXM, CN, DO) (T, CPD, C, CXM, PEF, DO, AMC) (CPD, C, CXM, CRO, PEF, CN, DO, IPM) (T, CPD, C, CXM, CRO, PEF, CN, DO, IPM, AMC) with corresponding multiple antibiotics resistance index of (0.4%, 0.5%, 0.6%, 0.7%, 0.8%,1.0%) respectively.

Table 3, shows the frequency of occurrence of antibiotic resistance genes in the multidrug resistant *E. coli* strain. The *tetA* gene was detected in 10(76.9%) *E. coli* isolates from Madara, 5(100%) Kindrimo, 3(60%) from Nono and 4(80%) from Manshanu. The *tetA* gene had the highest frequency of occurrence of 22(78.6%) in the *E. coli* isolates. Followed by *dfrAI* gene which was detected in 5(38.5%) of *E. coli* isolates from Madara, 3(60%) Nono and 1(20%) Manshanu with the frequency of occurrence of *dfrA1* gene 9(32.1%). The *blaCITM* gene was detected in 1(7.7%) of *E. coli* isolate from Madara, Kindrimo and Manshanu 1(20%) each, with total frequency of occurrence of 3(10.7%). The *blaSHV* gene was detected in 3(23.1%) from Madara with frequency of 3(10.7%) while *blaTEM* gene was detected in 1(7.7%) of *E. coli* isolates from Madara with frequency of 1(3.6%).

The *tetB* gene was detected in 1(20%) from Kindrimo with frequency of occurrence of 1(3.6%). Whereas *qnr* and *aac (3)-iv* gene 0(0%) each were not detected in the *E. coli* isolates.

Fig. 1 shows that *tetA* gene (577bp) was detected in *E.coli* isolate depicted in lanes 1-7, 9, 11, 12, 13 and 14. The *tetB* gene (634bp) was detected in *E. coli* in lane 12, *blaSHV* gene (768bp) was shown in lane 3, *blaCITM* gene (462bp) was detected in *E. coli* isolates identified in lanes 5 and14, *blaTEM* gene (857bp) in the *E.coli* strain was shown in lane 4 and *E. coli* isolates in lanes 2, 4, 12 and 13 were shown to carry *dfrA1* gene (367bp).

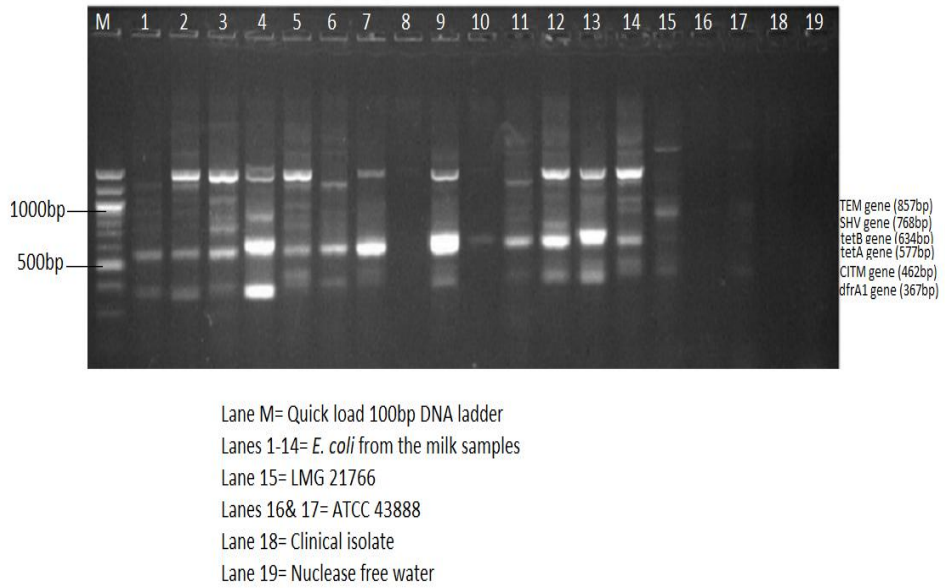

Fig. 1. Agarose gel electrophoresis of antibiotics resistance genes by multiplex PCR

4. DISCUSSION

This study revealed that the *E. coli* isolates found in the raw cow milk and milk products showed the presence of antibiotics resistance genes with several resistance patterns. *Escherichia coli* is a food pathogen often present in raw milk of animal origin. These finding agree with the reports of Coastard et al. [36], Kuehn [37] and Hasan et al. [38].

The raw cow milk and its products are often peddled in an unorganized manner that may be a source of bacterial contamination resulting from unhygienic practices during milking, the use of unpotable water, unclean utensils, unsafe environment and poor temperature storage [39].

The result revealed the presence of resistant *E. coli* in the milk samples. Similar findings were reported by Caudell et al. [40] that raw milk haboured resistant microbes. Sharma et al. [39] and Sobur et al. [41] reported that resistant *E. coli* were found in raw milk sold in India and Bangladesh respectively. The indiscriminate use and misuse of antibiotics in animals for therapeutic and prophylactic purposes and also as growth promoters in food producing animals has led to selective pressure on antibiotic resistant microbes. This has led to an increase in population and expression of resistant microbes [42] in food of animal origin. *Escherichia coli* is commonly found in the gastrointestinal tracts of animals. They serve as an indicators of antibiotics resistance showing selective pressure of one antibiotics to the other and are capable of transferring and acquiring resistance genetic elements [43].

The *E. coli* isolates showed multiple phenotypic antibiotic resistance to several antibiotics, Cefpodoxime (CPD) (100%), Cefuroxime Sodium (CXM) (100%), Chloramphenicol (C) (100%), Amoxicillin (62.9%), Tetracycline (54.3%) and Doxycycline (40%) respectively. The finding agrees with the report of Sobur et al. [41], that *E. coli* isolated from raw milk showed high resistance to tetracycline (89.44%), oxytetracycline (78.89%), Hossain et al. [44] reported tetracycline resistance (70%), amoxicillin resistance (84%) and Sharma et al.[39] reported tetracycline resistance of (33.34%). Jaja et al.[45] reported that the *E. coli* isolated from meat in South Africa showed resistance to Chloramphenicol (24.5%), Ceftrioxone (20.6%).

On the contrary, Sharma et al. [39] reported that the *E. coli* isolates were sensitive to chloramphenicol (85.96%), cephalosporins (Cefotaxime) (85.96%) and Cephalexine (64.91%). The variations in the sensitivity pattern could be attributed to the concentrations and frequency of usage of these antibiotics in food animals at the different geographical locations.

The finding in this study showed that the *E. coli* isolates were sensitive to gentamicin (88.6%), imipenem (88.6%), perfloxacine (80%) and ceftriaxone (68. 6%). This is similar to the report of Hossain et al. [44] where the *E. coli* isolates were sensitive to gentamicin (100%) and ciprofloxacin (89%). Although, the *E. coli* isolates were mildly resistant to quinolones, aminoglycosides and carbapenems in a previous study by Yu et al. [46] in China.

Contrastingly, Sobur et al. [41] reported that *E. coli* from raw milk showed (66.67%) resistance to ertepenem. This could be attributed to the transmission of carbapernem resistant bacteria from farm workers and visitors to the animal farm environment. The carbapernem drugs (imipenem, and ertepenem) are mostly last resort drugs used to treat fastidious multidrug resistant *E. coli* infection in humans. Carbapenem-resistant Enterobactericeae have been grouped as critical priority pathogens by WHO [47].

The *E. coli* isolates showed MDR phenotypes to at least three classes of antibiotics (penicillins, cepholosporins, tetracyclines and amphenicol). The MDR phenotype pattern ranged between four to ten MDR patterns. Beta-lactams, tetracycline and amphenicols were the most frequent multidrug resistance patterns and this agrees with the work of Hossain et al. [44]. This suggests that the raw cow milk and its products is a high risk food that could possibly transfer resistant food pathogens to humans through consumption [45]. Worldwide, the consumption of raw milk [40] in both developed [48, 27] and undeveloped countries is on the rise because of the health benefits to be derived. This implies rise in the acquisition and spread of antibiotics resistance genes in humans, not surprising there is a global increasing prevalence of antibiotic resistance [49].

The *E. coli* isolates in this study exhibited co-resistance to certain antibiotics. Similar findings were reported by Hossain et al. [44], and Yu et al. [46]. The *E. coli* isolate is capable to co-resist

more than one antibiotics by different resistance mechanisms.

Some *E. coli* isolates in this study 22 (78.6%) were positive for the tetracycline resistance gene (*tetA*) encoding the Tetracycline resistance. The *tetA* resistance gene was the predominant resistance gene in the raw milk and its products followed by *dfrA1* (9, 32.1%), *blaSHV* and *bla CITM* (3, 10.7%), *blaTEM* and *tetB* (1, 3.6%). While *qnr* and aac(3)iv were not detected. The finding is similar to the report of Sobur et al. [41] that *tetA* gene was the most prevalent resistance gene detected in the milk samples.

Indiscriminate and prolonged use of tetracycline and its derivatives as growth promoters and prophylaxis in food-producing animals could be responsible for the high prevalence of *tetA* gene [50].

The identification of a high proportion of *tetA* genes in TET-resistant isolates indicates that the main mechanism of TET resistance in *E. coli* isolates is possibly through active efflux pump [51]. The *tetA* resistance gene is widespread among the members of Enterobactriaceae and the resistance genes are plasmid mediated and possibly transferred to other commensals and pathogenic bacteria by conjugation [52].

The study showed that the *E. coli* isolates were positive for resistance genes (*tetB*) of 1(3.6%) and this finding agrees with the reports of Sobur et al. [41] that detected *tetB* gene in the *E.coli* isolate 9(37.5%) from cow milk. The variation in the frequency of occurrence of *tetB* could be attributed to the antibiotics usage in food producing animals and method of detection.

The *E. coli* isolates were positive for β-lactamase resistance genes as follows *blaSHV* (3, 10.9%), *blaCITM* (3, 10.9%) and *blaTEM* (1, 3.6%). The result is consistent with the reports of Momtaz et al. [53], *bla SHV* (6.38%), *blaCITM* (12.76%), [54] *blaSHV* (38%), *blaCITM* (36%), [55] *blaTEM* (56%) and *blaSHV* (4%) and [56] 49(59%) *blaTEM* were reported. Yu et al. [46] reported that *blaTEM* resistance genes was the prevalent resistance genes detected in *E.coli* isolate from cow milk in China.

Overuse of β-lactam drugs in food producing animals, could lead to selective pressure in *E. coli* isolates to acquire resistance determinants against β-lactam drugs [57,58]. In contrast [14] in Switzerland there was no β-lactamase resistance genes found in the milk examined, the prudent use of antibiotics and standard hygienic measures in the area could account for absence or non-expression of the resistance genes.

The resistance genes (*dfrA1*) encoding plasmid-mediated resistance to trimethoprim was detected in 9(32.1%) of *E. coli* isolates which agrees with the reports of [53,59,58] (Iran, China and Burkina Faso) where *dfrA1* genes were detected in the *E coli* isolate from milk products.

The quinolones resistance genes (*qnr*) was not detected in the *E. coli* isolates, this result is consistent with the report in Korea [56] where there was little or no usage of quinolones in the study area [60]. Quinolones may not be commonly used in food-producing animals in Nigeria indicating low prevalence of resistance phenotype even also the absence of quinolone resistance genes. Though it is possible that susceptible isolates may carry resistance genes that are not expressed [61].

The resistance gene of (*aac(3)iv*) encoding resistance to aminoglycosides (gentamicin) were not present in the study. On the contrary [53, 62, 54, 63] (27.65%, 64.17%, 32%, 94.44%) respectively, reported that the *E. coli* isolates carried the resistance genes (*aac(3)iv*). The high values could be attributed to indiscriminate use of gentamicin in food-producing animals in those areas which causes selective pressure in the zoonotic or commensal bacteria to acquire resistance against aminoglycosides. Polymerase Chain Reaction probe is a vital tool in the identification of genetic determinants in microbes, however positive PCR signal does not always detect the presence of a functional resistance gene as other resistance genes could be present. It is possible that isolates with phenotypic resistance, that did not express resistance genes may have carried another gene or mutant form [56]. Therefore necessitate that even susceptible isolates could be subjected to molecular studies.

5. CONCLUSION

The study showed that multidrug resistant *E. coli* haboured antibiotics resistance genes namely *tetA*, *tetB*, *dfrA1*, *blaTEM*, *blaCITM* and *blaSHV* that were screened for. This indicates that the raw cow milk and its products are high risk foods when consumed and could possibly transfer multidrug resistant bacteria in humans leading to prolonged treatment without cure. Continuous

food surveillance programs that will routinely monitor and screen these foods and stringent regulations of the use of antibiotics in food producing animals is expedient in order to curb the menace of antibiotics resistance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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