Investigation of Extended Spectrum Beta-Lactamase Producing *Escherichia coli* and other Cefotaxime-Resistant Bacteria in Cow Milk in Nigeria

Amosun, E. A.,[1] Ogunbadewa, A. J.,[1] Ojo, O. E.[2*] and Akinade, S.A.[3]

[1] Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria.
[2*]Department of Veterinary Microbiology, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Abeokuta, Nigeria.
[3] FrieslandCampina Wamco Nigeria PLC, Ogba, Lagos State, Nigeria.

* **Corresponding Author:** Professor Ojo, Olufemi E., Department of Veterinary Microbiology, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria. Tel.: +234 703 0425 778. *E-mail address:* ojooe@funaab.edu.ng

ABSTRACT

Production of extended spectrum β-lactamases (ESBLs) confers resistance to cefotaxime as well as many third and fourth generation cephalosporins in bacteria. ESBL-producing *E. coli* strains have been implicated in refractory infections in humans and dairy cows in Nigeria. The present study investigated the presence of cefotaxime-resistant bacteria including ESBL-producing *E. coli* in the milk of apparently healthy cows from smallholder dairy herds. A total of 168 non-duplicate milk samples were collected from 34 cattle herds in three local government areas of Oyo State, Nigeria. Cefotaxime-resistant bacteria were isolated from milk samples by selective culture on MacConkey agar supplemented with cefotaxime (1mg/L). Isolates were identified by biochemical tests. Phenotypic ESBL production was determined using cefpodoxime/cefpodoxime-clavulanic acid combination discs. Presence of ESBL genes (*bla*$_{\text{TEM}}$, *bla*$_{\text{SHV}}$ and *bla*$_{\text{CTX}}$) and phylogenetic groups (A, B1, B2, C, D, E, and F) of *E. coli* were determined by polymerase chain reaction assay. Susceptibility to other antimicrobials was carried out using the Kirby-Bauer disk diffusion method. Cefotaxime-resistant bacteria were detected in milk samples of in 40 (23.8%) out of 168 individual cows. At least one sample from 19 (55.9%) out of 34 herds yielded a cefotaxime-resistant isolate. The isolates were identified as *Escherichia coli* (9/168; 5.4%), *Enterobacter amnigenus* (8/168; 4.8%) and *Pseudomonas aeruginosa* (23/168; 13.7%). Five (3.0%) of all 168 samples were positive for phenotypic ESBL-producing bacteria. All the ESBL-producing bacteria were *E. coli* and possessed *bla*$_{\text{CTX-M}}$ which is of the *bla*$_{\text{CTX-M-15}}$ gene variant. Two isolates also possessed the *bla*$_{\text{TEM}}$ gene. ESBL-producing *E. coli* isolates belonged to phylogenetic groups A (n=3) and B1 (n=2). Overall, the cefotaxime-resistant bacteria showed 100.0% resistance to ampicillin and cefotaxime, 90.0% to tetracycline, 72.2% to amoxicillin/clavulanic acid, 47.5% to streptomycin, 40.0% to sulphamethoxazole/trimethoprim, 37.5% to chloramphenicol, 32.5% to ceftazidime and 10.0% to each of ciprofloxacin and gentamicin.

Keywords: ESBL Resistance Genes; Cow Milk; Cefotaxime-Resistance; *Escherichia coli*.

INTRODUCTION

The use of antimicrobials agents has helped in the prevention and control of infections in humans and animals (1). However, the emergence and spread of antimicrobial resistant bacterial strains is a major global threat to public and animal health and food security with great socioeconomic implications (2, 3). The increasing reports of antimicrobial resistance in bacterial strains from diverse human, animal, environmen-

tal and food sources show the wide range of distribution, ease of transmission and enormity of the danger associated with these organisms (2, 4, 5). Some have predicted that the world is gradually approaching the end of the 'antibiotic era' with dire consequences for health care delivery (6, 7).

Cefotaxime is an important third generation cephalosporin developed in response to the challenges associated with treatment of infections caused by bacteria showing resistance to earlier generations of cephalosporins and penicillins (8). Cefotaxime is a broad-spectrum β-lactam with activity against Gram negative and some Gram-positive bacteria (9, 10). The β-lactam antimicrobials are among the most widely used antimicrobial agents in human and veterinary practices because of their relatively high efficacy, wide safety margin and low cost (11). Resistance to cefotaxime was soon noticed following its introduction for clinical use (12). Since then, the trend has contined.

Production of cefotaximase otherwise known as the CTX-M type β-lactamase is one of the mechanisms by which bacteria develop resistance to cefotaxime (13). Cefotaximase is a type of extended spectrum β-lactamase (ESBL), which is capable of hydrolyzing and inactivating a wide range of β-lactams. Bacteria that produce the CTX-M type ESBL appeared to be more successful than producers of the other ESBL types in their dissemination across wide geographical locations (14). The CTX-M β-lactamase is the most commonly encountered ESBL type in bacterial isolates from clinical samples (13).

Escherichia coli is among the most commonly encountered bacteria in clinical samples both in humans and in animals (11). It is associated with intestinal and extra-intestinal infections including urogenital tracts infections and septicaemia. Strains of *E. coli* occupy diverse habitats where they may exist as commensal organisms, opportunistic pathogens or primary pathogens. Hence, phylogenetic classification is very useful in determining the ecological niche, lifestyle and pathogenic potentials of *E. coli* isolates originating from different sources (15).

E. coli can be transmitted from animals to humans through consumption of contaminated milk and contact with dairy cows (16, 17, 18). Milk is a good source of protein for humans but also a rich medium for bacterial proliferation (19). Nigeria depends more on importation of milk because local production is grossly insufficient to meet the current demand. In recent years, the Nigerian Government in partnership with foreign private dairy developers has been working to improve the quality and quantity of local milk production. The targeted producers are smallholder farmers with low level of formal education. The local smallholder dairy farmers and milk processors do not operate under strict hygiene. Therefore, milk could be contaminated with pathogens, which may lead to serious infection in consumers. It is expected that government intervention will help to boost the quantity of milk produced by local farmers as well as improve the quality of locally produced milk to international standards.

This study investigated the occurrence of ESBL-producing *E. coli* and other cefotaxime-resistant bacteria in milk samples from selected dairy herds of smallholder farmers that were participating in the government intervention program in Oyo State, Nigeria. In addition, the resistance of the bacterial strains to other antimicrobial agents, the presence of ESBL resistance genes and phylogenetic groups of ESBL-producing *E. coli* were determined.

MATERIALS AND METHODS

Sample collection

Before collection of milk samples, the health status of the cows was determined by physical examination done by qualified practicing veterinarians. The udder and milk samples were examined for physical abnormalities. Clinically sick animals were excluded from the study. Subclinical infection were not determined.

Milk samples were collected from 34 cattle herds in Iseyin (9 herds), Ibarapa East (10 herds) and Itesiwaju (15 herds) Local Government Areas of Oyo State. One hundred and sixty-eight milk samples were collected from these herds. Five samples were collected from each herd except in one herd where three samples were collected. Before collection the udder including the teats of every cow was thoroughly disinfected by washing with soapy warm water, drying with paper towel and disinfection using methylated spirit soaked in cotton wool. Mid-stream milk sample was collected into universal bottles from individual lactating cows. All the cows investigated in this study were of the White Fulani breed and they were between 3 to 7 years old. Milk samples were collected during the usual hand-milking operation carried out by farmers for commercial milk collec-

tion from the herd. Samples were labelled appropriately and transported to the laboratory (in icepacks) for immediate microbiological analysis.

Isolation and identification of cefotaxime-resistant Enterobacteriaceae

One millilitre of milk sample was inoculated into nine milliliters of buffered peptone water (BPW) and incubated at 37°C for 16–18 hours for pre-enrichment. Afterwards, a loopful of the pre-enrichment broth was streaked on MacConkey agar (with ampicillin supplement at 100mg/) and incubated at 37°C overnight (20). Discrete colonies of lactose and non-lactose fermenting bacteria of were selected and inoculated onto MacConkey agar supplemented with cefotaxime at 1mg/L for selective isolation of cefotaxime-resistant isolates (20). The inoculated plates were incubated at incubated at 37°C for 18–24 hours. Colonies of cefotaxime-resistant isolates were selected, purified and preserved on nutrient agar slopes for further investigation. Selected isolates were subjected to biochemical characterization for species identification using commercial biochemical identification kit (Oxoid™ Microbact™ GNB 24E) and computer-aided interpretation.

Phenotypic detection of extended spectrum β-lactamase producing isolates

All cefotaxime-resistant isolates were tested for phenotypic detection of extended spectrum β-lactamase production. The phenotypic ESBL production test was carried out using the cefpodoxime/cefpodoxime-clavulanic acid combination disk test kit (Oxoid™, ThermoFischer Scientific) as previously described by Okpara *et al* (21). Fresh colonies of test organism were emulsified in normal saline at a turbidity equivalent to 0.5 McFarland standard. The bacterial suspension in normal saline was spread evenly on Mueller Hinton agar (MHA) after which cefpodoxime (CPD10, 10 µg) and cefpodoxime-clavulanic acid (CD01, 10/1 µg) were firmly placed on the agar. The test plates were incubated at 35±2°C for 16 – 18 hours. The diameter of the zone of inhibition around each disk was measured. The difference between the measurements of diameter of the zones of inhibition round both disks was determined. Isolates with a difference of five millimeter and above in the zones of inhibition between the two disks was classified as phenotypic ESBL producer.

Determination of genetic basis of ESBL production and *E. coli* phylogrouping

All phenotypic ESBL-producers were screened for the detection of three major types of ESBL-associated genes (*bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{CTX-M}$) by polymerase chain reaction (PCR) assay using previously describe specific primers and conditions (Table 1). Genomic DNA was extracted from isolates by thermolysis (20). Briefly, 1 mL of overnight broth culture of test isolate in Luria-Bertani (LB) broth was heated in thermal block at 95 °C for ten minutes. After heating, the preparation was quickly transferred onto ice for cold shock. It was then cold centrifuged at 11,0000g for five minutes. The supernatant was collected as DNA extract while the residual cell sediment was discarded. The quality and concentration of DNA were measured by spectrophotometry (Thermo Scientific NanoDrop^{R} 1000 spectrophotometer). PCR assay was carried out using previously described primers and conditions (23, 24). The *bla*$_{CTX-M}$ gene variants were determined by nucleotide sequencing and sequence analysis as previously described by Okpara *et al* (21). In addition, ESBL-producing *E. coli* isolates were assigned to one of seven *E. coli sensu stricto* phylogenetic groups (A, B1, B2, C, D, E, and F) using a PCR-based method described by Clermont and colleagues (15) (Table 1).

Antimicrobial susceptibility testing

Cefotaxime-resistant isolates were tested for susceptibility to selected antimicrobial agents using Kirby Bauer disk diffusion methods in line with the recommendation of Clinical Laboratory Standards Institute (24). The following antimicrobial disks were included in the test: ampicillin (AMP, 10µg), amoxicillin/clavulanic acid (AMC, 20/10µg), cefotaxime (CTX, 5µg), ceftazidime (CAZ, 10µg), chloramphenicol (CHL, 30µg), ciprofloxacin (CIP, 5µg), gentamicin (GEN, 10 µg), streptomycin (STR, 10µg), tetracycline (TET, 30µg) and trimethoprim/sulphamethoxazole (SXT, 1.25/23.75µg). Isolates were tested for susceptibility to antimicrobial agents on Mueller Hinton agar using bacterial suspension (in normal saline) with turbidity corresponding to 0.5 McFarland standard. Inoculated MHA plates were incubated at 35±2°C for 16 – 18 hours. The diameter of zone of inhibition around each of the antimicrobial disks was measured to the nearest millimeter and results interpreted using the breakpoints stipulated by Clinical and Laboratory Standards Institute

Table 1: Primers for the detection of ESBL resistance genes and determination of *E. coli* phylogroups

Primers	Primers sequence (5'- 3')	Annealing Temperature (OC)	Target	Product size (bp)
ESBL resistance genes (Culik *et al*., 2010) (23)				
SHV-fw SHV-rv	TTCGCCTGTGTATTATCTCC TCCGCTCTGCTTTGTTATTC	55 55	*bla*SHV	750
TEM-fw TEM-rv	ATGAGTATTCAACATTTCCG TTAATCAGTGAGGCACCTAT	55 55	*bla*TEM	964
CTX-M-fw CTX-M-rv	CGCTTTGCGATGTGCAG ACCGCGATATCGTTGGT	55 55	Group1 *bla*CTX-M	551
CTX-M-9-fw CTX-M-9-rv	GCAGTACAGCGACAATACCG TATCATTGGTGGTGCCGTAG	55 55	Group 9 *bla*CTX-M	356
***E. coli* phylogrouping (Clermont *et al*, 2013) (15)**				
AceK.f ArpA1.r	AACGCTATTCGCCAGCTTGC TCTCCCCATACCGTACGCTA	59 59	*arpA*	400
chuA.1b chuA.2	ATGGTACCGGACGAACCAAC TGCCGCCAGTACCAAAGACA	59 59	*chuA*	288
yjaA.1b yjaA.2b	CAAACGTGAAGTGTCAGGAG AATGCGTTCCTCAACCTGTG	59 59	*yjaA*	211
TspE4C2.1a TspE4C2.2b	CACTATTCGTAAGGTCATCC AGTTTATCGCTGCGGGTCGC	59 59	TspE4.C2	152
trpAgpC.1 trpAgpC.2	AGTTTTATGCCCAGTGCGAG TCTGCGCCGGTCACGCCC	57 57	*trpA* (Phylogroup C confirmation)	219
trpBA.f trpBA.r	CGGCGATAAAGACATCTTCAC GCAACGCGGCCTGGCGGAAG	57 57	*trpA* (Internal control)	489

(24). *Escherichia coli* ATCC 25922 was included in the test for quality assurance.

Statistical analysis:

Data were expressed in absolute numbers and in percentages. The rates of detection of different bacteria species in cow milk and cefotaxime-resistant bacteria inherds among the Local Government Areas were compared using Chi square test with p value of <0.05 considered statistically significant.

RESULTS

Cefotaxime-resistant bacteria

Cefotaxime-resistant bacteria were detected in 40 (23.8%) of 168 milk samples. The organisms were present in five (11.1%) of 45 samples from Itesiwaju LGA, 17 (34.0%) of 50 samples from Iseyin LGA and 18 (24.7%) of 73 samples from Ibarapa East LGA (Table 2). Cefotaxime-resistant bacteria were identified as *Escherichia coli* (n=9), *Enterobacter amnigenus* (n=8) and *Pseudomonas aeruginosa* (n=23). There was no significant difference (p>0.05) in the rates of detection of the three bacteria species in milk samples. Cefotaxime-resistant bacteria were detected in 19 (55.9%) of 34 cattle herds visited across the three LGAs. Three (33.3%) of nine herds in Itesiwaju LGA, seven (70.0%) of 10 in Iseyin and nine (60.0%) of 15 in Ibarapa East were positive for cefotaxime-resistant bacteria. The number of positive herds was significantly lower (p<0.05) in Itesiwaju LGA than in Iseyin and Ibarapa East LGAs.

E. coli producing extended spectrum β-lactamase

Five of the cefotaxime-resistant *E. coli* isolates tested positive for phenotypic production of extended spectrum β-lactamase while other isolates did not produce ESBL. Thus, the rate of detection of ESBL-producing bacteria in all the 168 milk samples was 3.0%. All the five ESBL-producing *E. coli* were from separate herds. Four of the ESBL-producing *E. coli* isolates were from four herds within Iseyin LGA while one isolate was from a herd in Ibarapa East LGA. All the

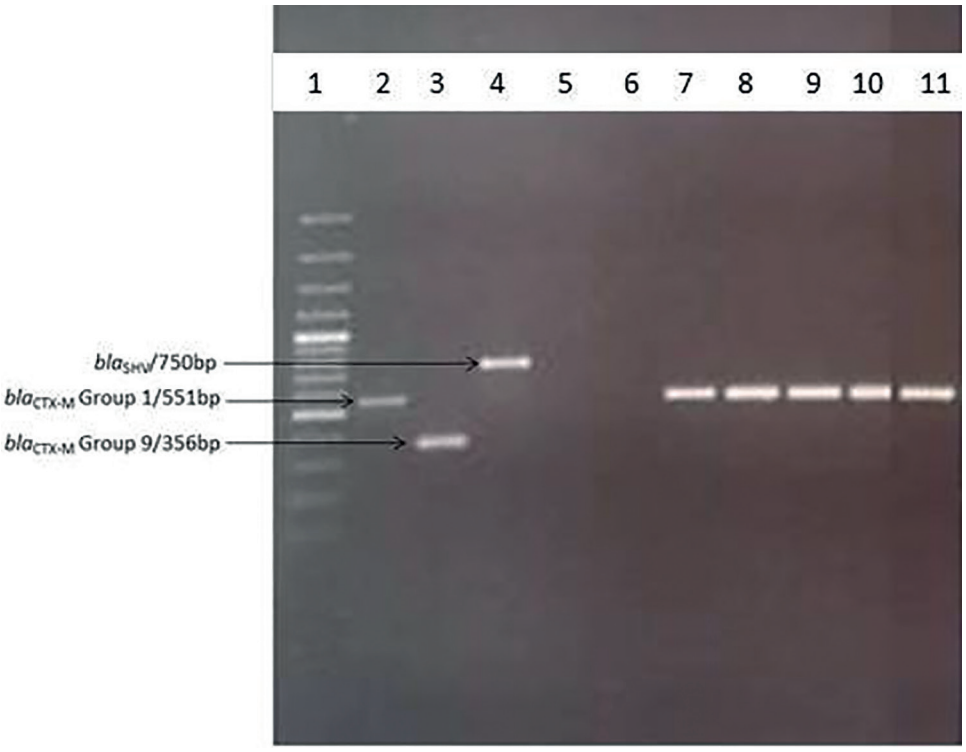

Figure 1: Agarose gel electrophoresis image showing bands of *bla*SHV, *bla*CTX-M-1 group and blaCTX-M-9 group detected by multiplex PCR assay. 1: Molecular marker, 2: *bla*CTX-M group 1 positive control, 3: *bla*CTX-M group 9 positive control, 4: *bla*SHV positive control, 5and 6: neggative controls, 7-11: *bla*CTX-M group 1 positive isolates

Table 2: Cefotaxime-resistant bacteria in milk samples from smallholder dairy farms

Local Government Areas	Sample Size	Number (%) of bacteria species from samples			Total
		E. coli	*Enterobacter amnigenus*	*Pseudomonas aeruginosa*	
Itesiwaju	45	1 (2.4)	2 (4.4)	2 (4.4)	5 (11.1)
Iseyin	50	5 (10.0)	5 (10.0)	7 (14.0)	17 (34.0)
Ibarapa East	73	3 (4.1)	1 (1.4)	14 (19.2)	18 (24.7)
Total	168	9 (5.4)	8 (4.8)	23 (13.7)	40 (23.8)

ESBL-producing isolates possessed the *bla*CTX-M-15 ESBL gene variant of *bla*CTX-M-1 group (Figure 1). In addition, two isolates possessed the *bla*TEM gene (Figure 2). One of the four isolates from Iseyin LGA as well as the only isolate from Ibarapa East LGA possessed *bla*TEM gene. The ESBL-producing *E. coli* isolates belonged to phylogenetic groups A (n=3) and B1 (n=2) (Table 3, Figure 3).

Antimicrobial Resistance

The *E. coli* isolates were all (100%) resistant to ampicillin and cefotaxime. In addition, they showed varying degrees of resistance to amoxicillin/clavulanic acid (55.6%), ceftazidime (66.7%), chloramphenicol (44.4%), ciprofloxacin (22.2%), gentamicin (22.2%), streptomycin (66.7%), sulphomethoxazole/trimethoprim (66.7%) and tetracycline (88.9%) (Table 4). Isolates of *Enterobacter amnigenus* demonstrated 100% resistance to ampicillin, cefotaxime and tetracycline. However, 62.5% of the isolates was resistant to amoxicillin/clavulanic acid, 12.5% to ceftazidime, 37.5% to chloramphenicol, 62.5% to streptomycin and 37.5% to sulphomethoxazole/trimethoprim. *Enterobacter amnigenus* isolates were all susceptible to ciprofloxacin and gentamicin (Table 4). Isolates of *Pseudomonas aeruginosa* were all (100.0%) resistant to ampicillin and cefotaxime but showed varying levels of resistance to other tested antimicrobials as follows: 82.6% to amoxicillin/clavulanic acid, 26.1% to ceftazidime, 34.8% to

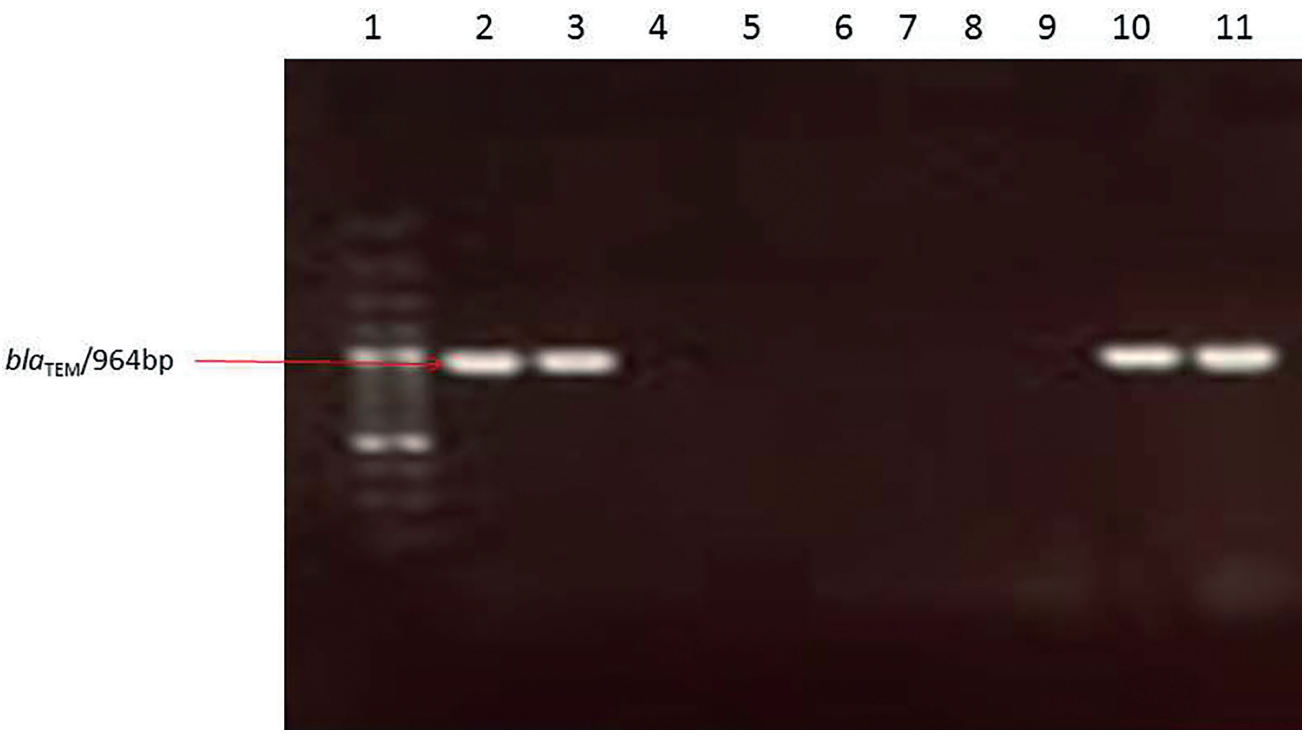

Figure 2: Agarose gel electrophoresis image showing bands of PCR amplified *bla*TEM ESBL resistance gene. 1: Molecular marker, 2 and 3: *bla*TEM positive control, 4 and 5: *bla*TEM negative control, 6-9: *bla*TEM negative isolates, 10 and 11: *bla*TEM positive isolates

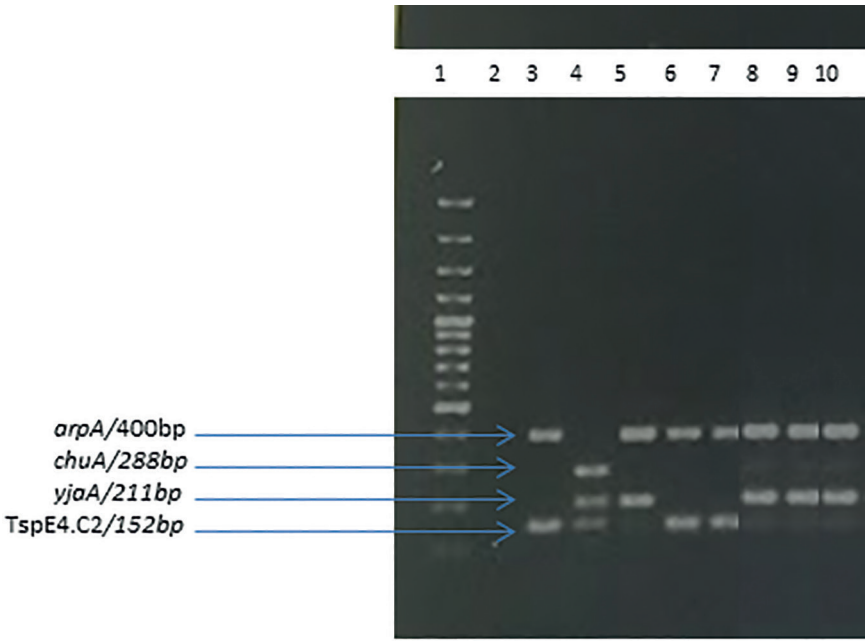

Figure 3: Agarose gel electrophoresis image showing different band combinations of *arpA*, *chuA*, *yjaA* and TspE4.C2 for assigning *E. coli* isolates into phylogroups in a multiplex PCR assay.1: Molecular marker, 2: Negative control, 3: Phylogroup B1 positive control (*arpA* and TspE4.C2), 4: Phylogroup B2 positive control (*chuA*, *yjaA* and TspE4.C2), 5: Phylogroup A positive control (*arpA* and *yjaA*), 6 and 7: phylogroup B1 positive isolates, 8-10: phylogroup A positive isolates.

Table 3: Characteristics of ESBL-producing *E. coli* from milk samples in Oyo State, Nigeria

Sample ID	Sample Source	Resistance gene	Phylogenetic group	Antimicrobial resistance profile
M48	Iseyin	*bla*CTX-M-15	B1	AMP-CAZ-CTX-STR-SXT-TET
M53	Iseyin	*bla*CTX-M-15, *bla*TEM	A	AMP-CAZ-CTX-SXT-TET
M95	Iseyin	*bla*CTX-M-15	A	AMP-CAZ-CTX-STR-SXT-TET
M100	Iseyin	*bla*CTX-M-15	B1	AMP-CAZ-CTX-TET
M161	Ibarapa	*bla*CTX-M-15, *bla*TEM	A	AMC-AMP- CAZ-CTX-SXT

Table 4: Antimicrobial resistance profile of cefotaxime-resistant bacteria in milk from smallholder dairy farms in Oyo State, Nigeria

Antimicrobial agents	Percentage of resistant isolates			Total (N = 40)
	E. coli (N = 9)	*Enterobacter amnigenus* (N = 8)	*Pseudomonas aeruginosa* (N=23)	
Ampicillin	100.0	100.0	100.0	100.0
Amoxycillin/clavulanic acid	55.6	62.5	82.6	72.2
Cefotaxime	100.0	100.0	100.0	100.0
Ceftazidime	66.7	12.5	26.1	32.5
Chloramphenicol	44.4	37.5	34.8	37.5
Ciprofloxacin	22.2	0.0	8.7	10.0
Gentamicin	22.2	0.0	8.7	10.0
Streptomycin	66.7	62.5	34.8	47.5
Sulphamethoxazole/trimethoprim	66.7	37.5	30.4	40.0
Tetracycline	88.9	100.0	86.9	90.0

chloramphenicol, 8.7% to each of ciprofloxacin and gentamicin, 34.8% to streptomycin, 30.4% to sulphomethoxazole/trimethoprim and 86.9% to tetracycline (Table 4).

DISCUSSION

With the increasing rate of emergence and spread of antimicrobial resistance among bacteria and the danger posed by this development to public health, it is of paramount importance to continuously monitor and identify potential sources of resistant bacteria (25). Food animals constitute huge reservoirs of resistant bacteria that can be disseminated in the environment and in animal-source foods (26). Therefore, surveillance for antimicrobial resistance in food animals and animal-source foods is critical as an integral part of the fight against antimicrobial resistance.

The present study showed a considerably high level of cefotaxime-resistant bacteria in fresh milk samples from smallholder dairy herds in Oyo State, Nigeria. The organisms were not limited to particular herds but were widely distributed across many herds in all the three LGAs investigated. It is generally considered that antimicrobial usage is the main driver of emergence of antimicrobial resistance in bacteria (27). Ceftiofur (a third generation cephalosporin) and cefquinome (a fourth generation cephalosporin) were developed for veterinary use and have been used for the treatment of infectious diseases in food animals (28). The use of these drugs has been implicated in the emergence of cefotaxime resistant bacteria in animals (29, 30).

In the present study, there was no evidence that ceftiofur or any other third generation cephalosporin had been used in the herds. The only beta lactam antimicrobial agent used in some of the herds as at the time of sample collection was penicillin. In an earlier study, the use of amoxicillin (a penicillin) was associated with the development of cephalosporin resistant *E. coli* in pigs (30). Another possible means by which positive cows could have acquired cefotaxime-resistant bacteria in their mammary gland is through direct contamination by resistant bacteria from faecal and environmental sources. The three bacteria species

(*E. coli, Enterobacter amnigenus* and *Pseudomonas aeruginos*a) identified in this study are part of the normal flora of the gut and could have easily gained access to the mammary gland through perineal soiling and contact with faecal material on the floor of the pen. Earlier authors have reported high levels of cefotaxime-resistant bacteria in the faecal and environmental samples from herds of dairy cattle (31). It is also important to note that the lactating cows were regularly hand-milked by the handlers. Cefotaxime-resistant bacteria could be introduced to the udder through human carriers involved in hand-milking. Other authors have also reported the detection of *E. coli, Enterobacter spp* and *Pseudomonas aeruginos*a in cow milk (32). These bacteria are not only of public health concerns but they are also of veterinary importance because of their association with disease conditions in animals. All the bacteria species identified in this study have been recognized as etiologies of clinical and subclinical mastitis in cattle (32). Mastitis is a major problem in the dairy industry because it could significantly increase the cost of milk production and lower the quality and quantity of milk yield (33). The involvement of antimicrobial resistant bacterial strains in infections could lead to prolonged morbidity and poor prognosis, increased spread of infection and heightened cost of treatment. There could be an exchange of bacterial pathogens between animals and humans through direct and indirect (contaminated environment) contacts as well as consumption of contaminated milk (34). Pathogenic *E. coli* strains could cause intestinal and extraintestinal infections in humans (35, 36). *Enterobacter amnigenus* has been recognized as major pathogen in many human clinical conditions (37, 38). *Pseudomonas aeruginosa* has been recovered from cases of nosocomial infections in humans, which oftentimes can be very difficult to manage because of resistance to antimicrobial agents (39, 40).

In the present study, five out of nine cefotaxime-resistant *E. coli* isolates were ESBL producers. The organism was detected in four out of 10 herds in Iseyin LGA as well as one out of 15 herds in Ibarapa East LGA. All five isolates possessed the *bla*$_{\text{CTX-M-15}}$ ESBL gene variant. The *bla*$_{\text{CTX-M-15}}$ is widely distributed globally and has been recognize to encode very potent enzyme that can hydrolyse a broad range of penicillins and cephalosporins including as well as third- and fourth- generation cephalosporins. Moreover, it is the most commonly encountered ESBL gene variant in clinical isolates of *E. coli* from humans. The *bla*$_{\text{CTX-M-15}}$ is detected in high frequency in ESBL producing *E. coli* of animal origin in Nigeria (20, 21, 41).

Finding from the present study confirm the presence of ESBL-producing bacteria in cow milk in Nigeria. The rate of detection of the organism was found to be low at 3.0%. Earlier authors have reported similar detection rates of 3.3% in Colombia (42) and 4.5% in Germany (43). However, a higher detection rate of 22.6% was reported in Turkey (44). The present study reports for the first time the presence of ESBL-producing *E. coli* from milk and cattle in Nigeria.

The detection of ESBL producing *E. coli* in milk is of veterinary and public health significance notwithstanding the low occurrence as observed in the present study. Previous studies have implicated ESBL-producing *E. coli* that possessed the *bla*$_{\text{CTX-M-15}}$ in cases of bovine mastitis (45). Moreover, these bacteria could be transmitted to humans through consumption of unpasteurized milk, a common practice among cattle herders (46). None of the cefotaxime-resistant *Enterobacter amnigenus* and *Pseudomonas aeruginosa* isolates produced ESBL. Overexpression of inducible chromosomally encoded AmpC beta-lactamase could produce cefotaxime-resistance in *Enterobacter* spp. (47, 48). Earlier authors reported *Enterobacter amnigenus* among species with natural susceptibility to cefotaxime (38). *Pseudomonas* species are known to possess many mechanisms that make them resistant to many antimicrobials (39). Besides enzymatic inactivation of antimicrobial agents, *Pseudomonas* species are known to possess efflux pumps as well as low level of permeability to β-lactams (39). Earlier studies on bacteria susceptibility to new generation cephalosporins showed that *Pseudomonas aeruginosa* had higher cefotaxime minimum inhibitory concentrations compared to other tested species (8). The present study did not investigate the mechanism of cefotaxime-resistance in the non-ESBL producing bacteria.

While striving to boost local milk production, great attention should be given to safety issues to circumvent potential health hazards associated with milk production. Improved herd hygiene, better udder healthcare and adoption of modern milking methods could prevent bacterial infection of the mammary gland and subsequent transfer of bacteria to milk. Consumption of unpasteurized milk should be discouraged through increased awareness and education of herders.

CONFLICT OF INTEREST STATEMENT

We did not receive funds for this work from any agencies and there is no conflict of interest to declare.

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