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Prevalence and antibiogram of coliform bacteria, and occurrence of fungi in subclinical mastitis in small ruminants in Plateau State, Nigeria

Kenneth Nnamdi Anueyiagu*, Solomon Kadiya Audu, Bature Davou Joshua, Olorungbemi Elijah Pelumi and Shehu Abdulazeez Haji

Federal College of Animal Health and Production Technology, NVRI Vom, Nigeria.

*Corresponding author. Email: anueyiagunnamdi@yahoo.com

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ABSTRACT: This study was conducted to determine the prevalence of coliform bacteria and fungi in ovine and caprine raw milk in Plateau State of Nigeria. In a cross sectional study, a total of 412 milk samples were collected aseptically and 206 questionnaires form where data such as breed, age, parity, lactation stage, floor type, and husbandry system were analyzed. Ewes and does without clinical mastitis were subjected to California Mastitis Test (CMT) to determine the presence of subclinical mastitis. Bacteriological assays and antibiotic susceptibility tests were performed according to standard guidelines. Fungal assays and identification were done according to standard protocol. The overall prevalence of subclinical mastitis for ewes and does were 28.2 and 35.8% respectively. Out of the risk factors examined, age and floor type showed statistically significant relationship with mastitis. Coliforms isolated from milk samples included *Escherichia coli, Klebsiella pneumoniae, Citrobacter freundii, Enterobacter aerogenes,* and *Serratia marcescens.* The highest coliform isolated among the ewes with 38% prevalence was *E. coli* and the least was *S. marcescens* with 2.5%. Among the does, *E coli* had the highest prevalence as well with 43.1% and *E. aerogenes* the least with 1.5%. *Aspergillus* species had 49 isolates out of the 94 fungal isolates (52.1%) of mycotic agents of mastitis in small ruminants. Most antibiotics used in this study showed extremely high level of antimicrobial resistance especially for Amoxicillin-clavulanic acid 45/79 (57.0%) and 53/65 (81.5%) in coliforms isolated from ewes and does respectively. The principle of one health approach which targets the environment, animals and humans should be considered important. Sensitization of pastoralists on good hygienic measures, and treatment of animals by qualified and registered veterinary personnel should be intensified.

Keywords: Antibiogram, coliform, does, ewes, fungi, mastitis, prevalence, udder infection.

INTRODUCTION

Mastitis is a complex inflammatory disease of mammary glands which could be chronic or fatal. In the livestock industry, it has been known to cause among many things poor fertility, increase premature culling rates, decrease in milk yield and quality, and increase in veterinary costs. In sheep and goats, mastitis is usually detected shortly after lambing and lasts till the weaning period. It can be categorized based on clinical manifestation and causative pathogens. Clinical manifestation can be grouped into clinical and subclinical mastitis. Clinical mastitis (CM) is the abnormality observed on the udder or milk of ruminants. The udder could show redness, swelling, high temperature or pain as reported by Fox (2009) and Mpatswenumugabo et al. (2017). In mild clinical mastitis, milk produced are characteristic of clots, changes in color or consistency, while in severe cases, fever, anorexia and/or shock may be observed. Subclinical mastitis (SCM) is the presence of infection without obvious signs. SCM has an elevated somatic cell count that is more than 200,000 cells/ml cut off point (Kandeel et al. 2017). This

type of mastitis can be more dangerous because it can persist for the entire lactation period or life of the animal. In addition, because it is asymptomatic, SCM could be ignored by farmers leading to economic losses due to low milk yield and quality and even culling of animals.

Mastitis due to causative pathogen can be contagious or environmental (Klaas and Zadoks, 2018). Contagious mastitis is caused by the spread of bacteria from infected udder to a healthy ruminant. The transfer of pathogenic bacteria from ruminant to ruminant takes place during milking either by the hands of pastoralists or milking machines. *Staphylococcus aureus, Streptococcus agalactiae* and *S. dysgalactiae* are examples of contagious pathogens. As the name suggests, environmental mastitis has its origin in the ruminant's environment such as soil, manure, water and bedding. The degree of cleanliness of the ruminant's environment determines to a large extent, the possibility of infection by environmental pathogens. Other predisposing factors such as poor management and injuries in teats can enable the entry of infectious diseases (Virdis et al. 2010). Apart from *S. aureus* which is the most frequently isolated pathogen in intramammary infection of sheep and goat, *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Corynebacterium* species and fungi such as *Aspergillus fumigatus* have also occurred (Seyedmousavi et al., 2015).

Coliforms are a group of *Enterobacteriaceae* that are gram-negative, rod-shaped, non-spore forming bacteria that ferment lactose with the production of acid and gas (Rudra and Dutta, 2018). They can be motile or non-motile. Coliforms are usually found in soil, water bodies and in large quantities in feces of warm-blooded animals. Their presence in food, water or milk is an indication of low hygienic standards. Coliforms such as *Escherichia coli*, *Klebsiella pneumoniae, Enterobacter aerogenes, Citrobacter spp,* and *Serratia spp* have been isolated from raw milk of small ruminants (Salman and Hamad, 2011). They could cause as many as 30 to 40% prevalence of clinical mastitis in a farm (Rudra and Dutta, 2018).

Besides bacteria, fungi can be found in the bedding and other utensils used in the environment and often lead to occasional outbreaks in the animals (Al-Muhna, 2017). Mastitis is caused by more than 26 species of fungi in small ruminants (Al-Muhna, 2017). Yeasts are the usual cause of both ovine and caprine mycotic mastitis but mastitis due to filamentous fungi like *Aspergillus* species has also been reported (Hassan et al., 2013). The increase in the occurrence of mycotic mastitis could lead to increase in resistance to antifungal drugs which might be of public health significance (Perez et al., 1998).

The treatment of mastitis in small ruminants due to coliforms is usually by intramammary infusion of antibiotics. However, misuse of antibiotics for treatment, control and prevention of diseases, and as growth factors, in ruminants is widespread in several places in Nigeria, a situation that provides a conducive atmosphere for the outbreak of zoonotic resistant bacterial infections in humans and livestock. Thus, the objective of this study was to determine the prevalence of coliform bacteria and their antibiogram, and occurrence of fungi in mastitis of small ruminants in Plateau State of Nigeria.

MATERIALS AND METHODS

Study area

Plateau State is located on an altitude of 1,200 meters between latitude 9.1667 and longitude 9.75. Plateau State has a near temperate climate with an average temperature of between 18 and 22°C with annual rainfall range of 1,300 to 1,500 mm (Plateau State Government, 2019).

The selection of ruminants' farms relied on different parameters including the convenience, accessibility, and verbal consent of the pastoralists to participate in the study.

Study design

A cross-sectional survey was carried out from July 2018 to July 2019 on lactating ewes and does in four Local Government Areas (Jos North, Barkin Ladi, Kanam, and Qua'anpan) of Plateau State which were conveniently selected out of 17 LGAs of the state.

Sampling and sample size determination

Ewes and does were selected randomly and only ruminants not involved in any antibiotic therapy regimen were screened for sample collection. The formula to calculate sample size of cross-sectional studies:

$$N = \frac{Z(pq)}{d^2}$$

Where N = Minimum sample size of coliform isolates of milk samples; Z = Standard error at 95% Confidence Interval (1.96); p = Local prevalence of 16.7% (Danmallam et al., 2018); q = 1 – p; d = degree of accuracy = 5% (0.05). The minimum sample size was 216 but 412 samples were collected.

A total of 206 well-structured questionnaires were administered face-to-face to collect livestock information including small ruminant's breed, age, parity, lactation stage, housing conditions, and general management conditions.

Physical examination and preparation of udder of study animals

Individual ewes and does were properly restrained

according to Mbuk et al. (2016) and clinically examined by a veterinarian. The shape, size, abnormal changes in milk, consistency, and temperature of udder, were findings that were considered a clinical mastitis case. The udder including the teats were washed with clean warm water and dried. The teats were thoroughly swabbed with cotton wool soaked in 70% ethanol before milk sample collection to remove dirt particles from the surface of the teats (Nibret et al., 2011).

California mastitis test (CMT)

Ewes and does which did not have clinical mastitis, were subjected to further investigation for subclinical mastitis by using California Mastitis Test (CMT) on milk samples from each half of sampled ruminant. It was carried out by adding equal amounts of CMT reagent and milk from each half on test paddle and was rocked for 10 seconds. Samples with a CMT score of 0 or T (trace) were considered negative while those with CMT scores of 1 (mild clumping), 2 (moderate clumping), or 3 (heavy clumping) were considered as positive for subclinical mastitis, according to Gebrewahid et al. (2012).

Sample collection

The 412 samples were collected by hand, first thing in the morning. The first stream of milk (foremilk) was discarded before 10 ml of milk from each half were aseptically collected into labelled sterile universal bottles (Zeryehun and Abera, 2017). The samples were kept at 4^{o}C in a cooler box and transported to the Microbiology Laboratory of Federal College of Animal Health and Production Technology Vom. Bacteriological examination was done according to Geser et al. (2012).

Laboratory isolation and identification of coliforms

One ml of each milk sample was inoculated into 9 ml of sterile peptone water for enrichment and incubated overnight at 37^{o}C. A loopful of the broth culture was streaked on sterile MacConkey Agar (Oxoid, UK) and Eosin Methylene Blue (EMB) Agar (Oxoid, UK) plates using the quadrant streaking method and incubated aerobically at 37^{o}C. The plates were checked for bacterial growth after 24, 48 and 72 hours to rule out slow growing bacteria. The colonies were examined for morphological features such as size, shape, and color. Pink colonies on MacConkey Agar, and greenish metallic sheen, purple, pink, blue-black, and orange colonies on EMB were subcultured respectively on freshly prepared MacConkey Agar and EMB Agar plates and incubated at 37^{o}C for 24 hours to get pure culture of coliform isolates.

Biochemical identification was carried out using standard conventional methods and Oxiod™ Microbact™ GNB 24E according to Mailafia et al. (2017). The presumptive Gram stained coliforms were subjected to conventional biochemical tests namely, Gelatin liquefaction, Nitrate reduction, Urease production, Oxidase, Indole-methyl-red-Voges-Proskauer (IMVP), Catalase, Citrate Agar, and Sugar fermentation tests (Müller et al., 2003). The confirmatory screening was carried out on presumptive Gram stained coliforms using Oxiod™ Microbact™ GNB 24E according to the manufacturers' instructions. About 1 to 3 isolated colonies were picked from an 18 to 24 hours culture and emulsify in 5.0 ml of sterile saline and mixed thoroughly to obtain a homogeneous suspension. The plate containing the substrates was placed in the holding tray and using a sterile Pasteur pipette, 4 drops (approximately 100 µl) of the bacterial suspension were added. Using a sterile pipette, the substrates underlined on the holding tray was overlaid with sterile mineral oil, in wells 1, 2, 3, and 24. However, wells 8 and 20 were not overlaid with oil for oxidase-positive, miscellaneous Gram-negative bacilli. Incubation was done at 37°C for 18 to 24 hours and results were read as described by the manufacturer. The steps of the procedure were followed as prescribed by Balows et al. (1991). Representative colonies were stored on slants of nutrient agar and kept in the refrigerator (4°C) until required for further work (David, 2011).

Laboratory isolation and identification of fungi

One ml of each milk sample was streaked in an 'L' shape on Sabouraud dextrose agar (SDA) and potatoes dextrose agar (PDA) plates which contained chloramphenicol (0.05 gm/l) and incubated separately at 25 and 37ºC for up to 7 days (Hassan et al., 2013). Fungi were stained with lactophenol cotton blue (David, 2011) and morphological characteristic identification were carried out according to Sarah et al. (2016). Individual colonies were picked and preserved on SDA slants (El-Sharoud et al., 2009).

Antibiotic sensitivity test

Antibiotic sensitivity test was carried out on the coliforms isolated using the disc diffusion method. The antibiotics employed were Ofloxacin (5 µg), Ciprofloxacin (5 µg), Amoxicillin/clavulanic acid (30 µg), Gentamycin (10 µg), Streptomycin (10 µg) and Pefloxacine (5 µg), produced by Thermo Scientific™ Oxoid™. These antibiotics were used firstly, because most of them were frequently used by pastoralist and secondly, because of their availability (Alhaji et al., 2019). Using sterilized wire loop, 3 to 5 colonies of the isolated coliforms from agar slants were inoculated into 4 ml sterile normal saline, the inoculum was standardized to 0.5 McFarland and incubated for 4 hours.

A sterile swab stick was dipped into the standardized inoculum and excess fluid removed from the swab by pressing it on the side of the bottle. The swab was used to spread on the entire surface of the dried Mueller Hinton agar plate. The plate was left on the bench for 20 to 30 minutes and then antibiotic discs were placed aseptically on its surface of the plate 15 mm apart. The plates were then incubated at 35 to 37°C for 18 to 24 hours. Thereafter, the diameter of the zone of inhibition around each antibiotic disc was measured in millimeters (mm) using a plastic transparent ruler and compared against a reference standard which contains measurement ranges and their equivalent qualitative categories of susceptible/sensitive (CLSI, 2014).

Ethical approval

Ethical approvals were obtained from village heads, heads of dairy companies and owners for ruminants that were sampled, and Ethical Research Committee of National Research Institute Vom, Plateau State.

Data analysis

The Statistical Package for Social Science (SPSS) version 23 software was used to analyze data collected. Each dairy ewe and doe or half-in-milking sampled was a unit of statistics. Ruminant-wise and half-wise mastitis were arranged in tabular forms. Risk factors data collected from questionnaires were analyzed and their statistical significance on the prevalence of ovine and caprine mastitis was calculated using chi-square ($\chi 2$) test. This is to find out the association between affected small ruminants and risk factors like breed, age, parity, lactation stage, floor type, and husbandry type. In addition, logistic regression was used to analyze and obtain odds ratio (with 95% confidence interval and $p < 0.05$ regarded as significant) to measure the degree of association between risk factors and the disease in small ruminants sampled. To build logistic regression which is dichotomous model, a lactating ewe or doe was defined as CMT positive if it had one half with a CMT score of 1+ or above, while all CMT scores of trace or negative were coded as 0 and all positive scores of +, ++, +++ were coded as 1. Results of bacteriological examination and susceptibility of coliforms isolated to antibiotics were arranged in tabular forms.

RESULTS

A total of 412 raw milk samples were collected from ewes and does in the four LGAs of Plateau State. There was no clinical mastitis recorded among the ewes. However, 28.2% of them were positive for subclinical mastitis (Table 1). Three halves of does showed visible signs of clinical mastitis giving 2.9% prevalence while 79 halves were positive for subclinical mastitis with 38.3% prevalence. In the half-wise prevalence**,** the does had more CMT positive percentages than the ewes (Table 2).

From the questionnaires collected, data from breed, age, parity, lactation, floor type, and husbandry system were studied. Age 1.0 to 1.11 years in both does and ewes had higher CMT positive result than the age 2.0 to 2.11 (Tables 3 and 4). Both animals were statistically significant for age; ewes ($p = 0.002$), does ($p = 0.039$). Does and ewes with two lambs had higher prevalence of subclinical mastitis than those with one or three. Early lactation in does had more cases of mastitis than the mid and late lactation stages, while among the ewes, mid lactation stage had more cases than the early and late (Tables 3 and 4). Nomadic system of rearing was more prevalent in does than the intensive system.

Coliforms isolated from raw milk of the ewes used in this study included *E coli, K. pneumoniae, C. freundii, E. aerogenes,* and *S. marcescens* with the highest being 38% for *E. coli* and the least being *S. marcescens* with 2.5% (Table 7). Among the does, *E coli* had the highest prevalence as well with 43.1% and *E. aerogenes* the least with 1.5% (Table 8).

When the coliform isolates were subjected to the following commonly used antibiotics: Ofloxacin, Pefloxacine, Gentamycin, Amoxicillin-clavulanic acid, Ciprofloxacin, and Streptomycin; isolates from ewes recorded Amoxicillin-clavulanic acid (57.0%) as the most resistant antimicrobial agents followed by Streptomycin (49.4%), Gentamycin (46.8%), Ofloxacin (44.3%), Pefloxacine (43.0%), and Ciprofloxacin (41.8%). Isolates from does recorded Amoxicillin-clavulanic acid (81.5%) as the most resistant antimicrobial agents followed by Pefloxacine (69.2%), Gentamycin (64.6%), Streptomycin (61.5%), Ofloxacin (58.5%), and Ciprofloxacin (52.3%).

DISCUSSION

This study showed that subclinical mastitis was more prevalent in ewes and does than clinical mastitis. The subclinical mastitis overall prevalence for ewes and does are 28.2 and 35.8% respectively (Table 1) which is in close agreement to the findings of Gebrewahid et al. (2012) where ewes had 28.14% prevalence but lower prevalence of 18.03% for does. However, Wakwoya et al. (2006) recorded 40.9% prevalence in does similar to what was discovered in this study. In this study, the does were most affected with subclinical mastitis than ewes. This may be due to genetic variation of the ewes and does bringing about sensitivity or otherwise to mastitis (Gebrewahid et al. 2012). It may also be due to the shorter dry period in does (Bergonier et al. 2003).

In this present study, there was no statistical significance in the effect of breed, parity, lactation stage, and floor type on the occurrence of subclinical mastitis in both the ewes

Table 1. Prevalence of mastitis at small ruminant and half levels in Plateau State, Nigeria.

Types of Mastitis	Small ruminant level (n = 103)		Half Level (n = 206)	
	No Positive	Prevalence	No Positive	Prevalence
Ewes				
Clinical	0	0	0	0
Subclinical	29	28.2	58	28.2
Does				
Clinical	3	2.9	3	1.5
Subclinical	38	35.8	79	38.3

Table 2. Half-wise prevalence of subclinical mastitis in lactating small ruminants in Plateau State, Nigeria.

Half	No tested	CMT positive No (%)		
		Trace	1+	2+
Ewes				
Left half	103	31(30.1)	14(13.6)	14(13.6)
Right half,	103	25(24.3)	21(20.4)	9(8.7)
Does				
Left half	103	22(21.4)	23(22.3)	18(17.5)
Right half	103	22(21.4)	21(20.4)	17(16.5)

Keys: 1+ = Somatic Cell Range (400,000 – 1,200,000), 2+ = Somatic Cell Range (1,200,000 – 5,000,000).

Table 3. Risk factors associated with ewes' mastitis in Plateau State, Nigeria.

Risk Factor	Category	No of animals examined	No of animals affected (CMT +ve)	Prevalence (%)	χ^2	p-value
Breed	Balami	103	58	56.3		
Age	1.0- 1.11 years	48	35	73.0	10.1	0.002**
	2.0-2.11 years	55	23	41.2		
Parity	Once	20	12	60.0		
	Twice	50	30	60.0	1.209	0.546
	Thrice	33	16	48.5		
Lactation stage	Early	41	23	56.1		
	Mid	39	24	61.5	1.107	0.575
	Late	23	11	47.8		
Floor type	Muddy	103	58	56.3		
	Concrete	0	0	0		
Husbandry system	Nomadic	103	58	56.3		
	Intensive	0	0	0		

and the does (Tables 3 and 4). This agrees with Gebrewahid et al. (2012). Age showed a statistical significance to the occurrence of subclinical mastitis in both ruminants, agreeing with the study of Beheshti et al. (2010) who indicated a relationship between age and subclinical mastitis of small ruminants with parity more

Table 4. Risk factors associated with does' mastitis in Plateau State, Nigeria.

Risk Factor	Category	No of animals examined (CMT +ve)	No of animals affected	Prevalence (%)	χ^2	p-value
Breed	Sokoto red	54	44	81.5	1.453	0.228
	WAD	49	35	71.4		
Age	1.0- 1.11 years	49	42	85.7	4.250	0.039*
	2.0-2.11 years	54	37	68.5		
Parity	Once	24	20	83.3	3.180	0.204
	Twice	48	33	68.8		
	Thrice	31	26	83.9		
Lactation stage	Early	44	31	70.5	2.372	0.305
	Mid	34	29	85.3		
	Late	25	19	76.0		
Floor type	Muddy	103	79	76.7		
	Concrete	0	0	0		
Husbandry system	Nomadic	47	47	100	26.262	0.000**
	Intensive	56	32	57.1		

Table 5. Association of risk factors with occurrence of mastitis in ewes in Plateau State Nigeria using logistic regression.

Risk factors	P value	Crude odds ratio (95% CI)	Adjusted odds ratio (95% CI)
Breed	0.000	5.787 (2.466 – 13.579)	2.478 (1.577 – 3.894)
Age	0.719	1.217 (0.553 – 2.679)	0.954 (0.738 – 1.233)
Parity	0.431	1.600 (0.691 – 3.707)	1.060 (0.916 – 1.227)
Lactation stage	0.037	3.418 (1.416 – 8.252)	1.114 (1.007 – 1.234)
Floor type	0.838	1.155 (0.528 – 2.527)	1.009 (0.930 – 1.094)
Husbandry system	0.680	0.985 (0.450 – 2.155)	0.984 (0.912 – 1.062)

Table 6. Association of risk factors with occurrence of mastitis in does in Plateau State Nigeria using Logistic regression.

Risk factors	P value	Crude odds ratio (95% CI)	Adjusted odds ratio (95% CI)
Breed	0.782	0.898 (0.356 – 2.265)	1.071 (0.659 – 1.742)
Age	0.000	0.811 (0.322 – 2.043)	1.894 (1.381 – 2.597)
Parity	0.023	1.456 (0.570 -3.718)	1.238 (1.030 – 1.488)
Lactation stage	0.556	2.256 (0.862 – 5.901)	0.959 (0.833 – 1.103)
Floor type	0.072	0.395 (0.107 – 1.460)	0.886 (0.776 – 1.011)
Husbandry system	0.015	1.049 (0.415 – 2.653)	1.133 (1.024 – 1.253)

than one. Mud floor type and nomadic husbandry system showed high prevalence of mastitis in the small ruminants. This meant that the breeding environments contribute to the contamination and exposure of teats to environmental pathogens. This agrees with Hogan et al. (1990) and Kurjogi and Kaliwal (2014) with similar results. This study showed that subclinical mastitis is more likely to occur in ewes with parity of more than one, early- and mid-lactation stages and muddy floor type. While in does, it is more likely to occur in Sokoto red than West African Dwarf, in younger age than older, parity of more than one and nomadic than intensive husbandry system.

In this study, coliforms isolated from raw milk from ewes and does were *E. coli, K. pneumoniae, C. freundii, E.*

Table 7. Coliforms isolated from ewes with mastitis in Plateau State, Nigeria.

Zone	No of +ve samples	Isolate (%)				
		E. Coli	*K. pneumoniae*	*C. freundii*	*E. aerogenes*	*S. marcescens*
Jos North	11	4(36.4)	4 (36.4)	2(18.1)	1(9.1)	0(0.0)
Barkin Ladi	12	5 (41.7)	4 (33.3)	1 (8.33)	2 (16.7)	0(0.0)
Kanam	17	8(47.1)	4(23.5)	5(29.4)	0(0.0)	0(0.0)
Qua'anpan	39	14(35.9)	13(33.3)	9(23.1)	1(2.6)	2(5.1)
Total	79	31(39.2)	25(31.7)	17(21.5)	4(5.1)	2(2.5)

Table 8. Coliforms isolated from does with mastitis in Plateau State, Nigeria.

Zone	No of +ve samples	Isolate (%)			
		E. Coli	*K. pneumoniae*	*C. freundii*	*E. aerogenes*
Jos North	10	5(50.0)	4(40.0)	1(10.0)	0(0.0)
Barkin Ladi	8	4(50.0)	3(37.5)	1(12.5)	0(0.0)
Kanam	17	7(41.2)	4(23.5)	5(29.4)	1(5.9)
Qua'anpan	30	12(36.7)	9(30.0)	9(30.0)	0(0.0)
Total	65	28(43.1)	20(30.8)	16(24.6)	1(1.5)

Table 9. Percentage of fungal species isolated from small ruminants in Plateau State.

Fungal pathogens	No of isolates	Percentage %
Yeast		
Candida famata	24	25.5
Mold		
Aspergillus flavus	17	18.1
Aspergillus fumigatus	3	3.2
Aspergillus niger	22	23.4
Aspergillus micheli	5	5.3
Aspergillus lentulus	2	2.1
Cladosporium cladosporioides	11	11.7
Microsphaeropsis arundinis	9	9.6
Microsporum audouinii	1	1.1
Total fungal isolates	94	100

Table 10. Percentage of resistant coliforms from ewes with mastitis to other antibiotics.

Coliforms	No +ve samples	OFX (%)	PEF (%)	CN (%)	AMC (%)	CPX (%)	S (%)
E. coli	31	16(51.6)	14(45.2)	16(51.6)	17(54.8)	13(42.0)	15(48.4)
E. aerogenes	4	4(100.0)	4(100.0)	4(100.0)	3(75.0)	3(75.0)	2(66.7)
S. marcescens	2	0(0.0)	0(0.0)	1(50.0)	2(100.0)	0(0.0)	1(50.0)
C. freundii	17	7(41.2)	8(47.0)	8(47.0)	9(53.0)	9(53.0)	8(47.0)
K. pneumoniae	25	9(36.0)	9(36.0)	9(36.0)	15(60.0)	9(36.0)	14(56.0)
Total	79	35(44.3)	34(43.0)	37(46.8)	45(57.0)	33(41.8)	39(49.4)

Key: OFX: Ofloxacin, PEF: Pefloxacine, CPX: Ciprofloxacin, AMC: Amoxillin-Clavulanic acid, CN: Gentamycin, S: Streptomycin.

aerogenes, and *S. marcescens.* Coliform bacteria are generally found in high concentrations in organic matter, such as beddings and manure (environment). Therefore, from an epidemiologic point of view, the primary source of infection for most pathogens in this study was environmental. Coliforms invade the udder through the teat

Table 11. Percentage of resistant coliforms from does with mastitis to other antibiotics.

Coliforms	No +ve samples	OFX (%)	PEF (%)	CN (%)	AMC (%)	CPX (%)	S (%)
E. coli	28	13(46.4)	16(57.1)	14(50.)	17(60.7)	12(42.9)	13(46.4)
E. aerogenes	1	1(100.0)	1(100.0)	1(100.0)	1(100.0)	1(100.0)	1(100.0)
K. pneumoniae	20	15(75.0)	13(65.0)	13(65.0)	20(100.0)	14(70)	15(75.0)
C. freundii	16	9(56.3)	15(93.8)	14(87.5)	15(56.3)	7(43.8)	11(68.8)
Total	65	38(58.5)	45(69.2)	42(64.6)	53(81.5)	34(52.3)	40(61.5)

Key: OFX: Ofloxacin, PEF: Pefloxacine, CPX: Ciprofloxacin, AMC: Amoxillin-Clavulanic acid, CN: Gentamycin, S: Streptomycin.

sphincter when teat ends come in contact with coliform bacteria. Once coliform bacteria enter the mammary gland, they either multiply rapidly or remain dormant. The highest coliform isolated among the ewes with 38% prevalence was *E. coli* and the least was *S. marcescens* with 2.5%. Among the does, *E coli* had the highest prevalence as well with 43.1% and *E. aerogenes* the least with 1.5% (Tables 7 and 8).

Pathogenic molds isolated from this study amounted to 74.5% while yeast had 25.5% which is similar to the work of Hassan et al. (2013) where molds had 60% (Table 9). Aspergillus species had 49 isolates out of the 94 fungal isolates resulting to 52.1% of mycotic agents of mastitis in small ruminants. This study is contrary to the findings of Hassan et al. (2013).

Qua'anpan had the highest number (39) of coliforms isolated from ewes while Jos North had the least (11); same with the isolates from does where Qua'anpan had the highest number (30) and the least was Barkin Ladi with 8. This could be due to high increase of heat and humidity in the Qua'anpan compared to the Kanam and Jos North. It is a fact that as heat and humidity increases, so does the bacterial multiplication and bacterial load in the environment. This is collaborated by a study done in India by Tiwari et al. (2013).

Most antibiotics used in this study showed extremely high level of Antimicrobial Resistance (AMR) more especially for Amoxicillin-clavulanic acid 45/79 (57.0%) and 53/65 (81.5%) in coliforms isolated from ewes and does respectively (Tables 10 and 11). Multidrug resistance (≥3 to 6 antibiotics) was seen in all coliform isolates especially *K. pneumoniae*, *C. freundii* and *E. coli*. Sawant et al. (2005) reported that beta-lactams and tetracycline were the most widely used antimicrobials on dairy animals. Hossain et al. (2017) reported a 100% resistance of *K. pneumoniae* to Penicillin, Cloxacillin, Streptomycin and Erythromycin. Same study reported a 98% resistance of *E. coli* to Cloxacillin, 96% to Penicillin, and 82% to Amoxicillin.

Conclusion

Subclinical mastitis is prevalent among ewes and does in Plateau State, Nigeria. Risk factors such as breed of ruminants, age, parity, lactation stage, and husbandry type are associated with the occurrence of mastitis among ewes and does. The study also revealed 5 coliforms (*E. coli, K. pneumoniae, C. fruendii, E. aerogenes,* and *S. marcescens*) and 9 fungi (*Candida famata, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus micheli, Aspergillus lentulus, Cladosporium cladosporioides, Microsphaeropsis arundinis, Microsporum audouinii)* which are associated with ovine and caprine mastitis in the State. Coliforms isolated have developed resistance against antibiotics currently used in animal treatment policies in Nigeria. Hence, the principle of one health approach which targets the environment, animals and humans should be considered here by maintaining good environmental conditions which assure the health of animals and the related quality of animal products used by humans. Sensitization of pastoralists on good hygienic measures, and treatment of animals by qualified and registered veterinary personnel should be intensified.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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