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Isolation and antibiotic susceptibility of bacteria from milk of apparently healthy goats in Abeokuta, Ogun State, Nigeria

**Olufemi, F. O., Odunfa, O. A., Akinduti, P. A. and keinde, O. B.
Department of Veterinary Microbiology & Parasitology
College of Veterinary Medicine, Federal University of Agriculture, Nigeria.
**woleoluemi84@gmail.com; +234 7080 488 340

Abstract

Milk contaminated with antibiotic resistant bacteria can be a major threat to public health. This study was conducted to investigate the antibiotic susceptibility of bacterial isolates from goat's milk in Abeokuta. Nine (9) bacteria species comprising 182 isolates were identified in 73 milk samples collected from six (6) different places located some 2 - 50 kilometers apart in Abeokuta. Isolation and identification of the bacteria species were carried out using standard microbiological procedures. The bacteria species were Pseudomonas spp (22.28%), Micrococcus spp (21.74%), Staphylococcus aureus (19.02%), Staphylococcus saprophyticus (15.22%), Enterobacter spp (8.70%), Bacillus spp (5.43%), Pasteurella spp (4.89%), Escherichia coli (2.17%), and Citrobacter spp (0.54%). Antibiotic susceptibility of the isolates and minimum inhibitory concentration were determined using a panel of 10 antibiotics by disc diffusion method and standard guidelines. The bacterial load from milk samples obtained in various locations are as follows:- DUFARMS, 0.1-1.2x10^{7}cfu/mL; Eweje, 0.6-1.2x10^{7}cfu/mL; Odeda, 0.6-3.5x10^{7}cfu/mL; Elite, 1.0-5.5x10^{7}cfu/mL; Elega, 0.1-2.52x10^{8}cfu/mL; and Obantoko 0.16-1.04x10^{8}cfu/mL. The mean counts were 0.54 ± 0.40x10^{7}cfu/mL; 0.78 ±0.13x10^{7}cfu/mL; 1.83±1.23x10 ^{7}cfu/mL; 2.58 ±1.45x10 ^{7}cfu/mL; 8.51 ± 5.60x10^{7}cfu/mL and 4.14 ± 3.90 x10 ^{7}cfu/mL respectively. Antibiotic susceptibility results showed that the organisms were 100% resistant to Amoxicillin,86.26% to Ceftriazone, 84.62% to Streptomycin, 82.42% to Chloramphenicol and 78.02% to Cotrimoxazol. However, the isolates were only 6.04% resistant to Ofloxacin and 11.54% to Pefloxacin suggesting that these might just be the only two antibiotics that the pathogens might respond to. In conclusion, microbes that are ordinarily commensals may be highly resistant to commonly used Antibiotics. This could pose serious problems in managing outbreaks associated these microbes. Reservoirs for bacterial resistance may be present in healthy animal populations and research is needed to accurately quantify the problem, propose and evaluate practicable solutions. There is the need to clarify the role of environmental factors, agents, and transmission of bacterial resistance in apparently healthy livestock.

Keywords: Milk, apparently healthy Goats, bacterial counts, Antibiotic resistance, Minimum Inhibitory Concentration, Abeokuta

Introduction

Goats play multiple roles in the livelihood of peasant households in Nigeria as it provides benefits in the form of meat, milk, manure, hide and skins and cash. Goats are highly adaptable to adverse environment (Peacock 2005) and they are a good source of petty income to the farmers in rural communities especially women and children. Goat's milk is highly nutritious and is superior to bovine milk on the ground of improved digestibility and higher buffering capacity and may have specific therapeutic value (Fillimon *et al.*, 2011). Goat's milk is less allergic than cow milk (Martin-Diana *et al.*, 2003). It is of specific economic interest in the developing countries because its production is one of the useful strategies to

stop the problems of poor nutrition in Africa and Asia (Guerrerio *et al*., 2013). This is because the milk either of goats, cow, or sheep contains aqueous colloidal suspension of nutritive proteins, fat and carbohydrates that contains numerous vitamins and minerals such as calcium, phosphorus, sodium, potassium and magnesium (Sangoyomi *et al.,* 2010). In addition the milk can be processed into numerous nutritious dairy products such as *wara, nono,* butter, yoghurt and cheese that are consumed by the local people.

However, Adesiyun *et al.* (2007) and Kagkli *et al.* (2007) have isolated some pathogenic organisms from fresh goat milk. The organisms isolated were *Listeria monocytogenes, Salmonella species, Compaylobacter spp., Bacillus cereus, Escherichia coli, Staphylococcus species, Streptococcus and Micrococcus spp*. Generally the microbial load count of these organisms is low in fresh Goat milk (Suguna *et al.*, 2011). However raw milk has low keeping quality and at room temperature, spontaneous microbial spoilage occurs turning the product sour within a few days. The growth and activities of these microorganisms in dairy food could make the food grossly unwholesome and harmful to consumers (Wouters *et al.*, 2002). Outbreaks of milk-borne diseases have occurred despite pasteurisation, as a result of either improper pasteurisation or product recontamination (Altekruse *et al.*, 1998; Nebedum and Obiakor, 2007). Therefore any factor that directly affects the quality of Goat milk and its dairy products is of public health or medical importance. Therefore for food safety reasons, microbiological analysis is carried-out routinely, to monitor and evaluate the level of prevalent pathogenic and spoilage microorganisms. Apart from the work of Ogbonna (2011), to our knowledge, there is a dearth of detailed reports on the microbiological quality of goat milk in Abeokuta, Ogun State, Nigeria. Hence, the main objective of this present study is to screen for the microbiological quality of fresh goat milk collected from randomly selected areas in Abeokuta. This is with a view to provide baseline information on the isolation, prevalence and characterization of pathogenic bacteria in milk of apparently healthy goats. The antibiogram of the microbial organisms and the minimum inhibitory concentrations of the antibiotics that are commonly prescribed in Nigeria that are in fresh milk collected from the udder of apparently healthy goats, are being investigated.

Materials and methods

This study was carried out in Abeokuta, capital of Ogun state in Southwest Nigeria situated at east bank of Ogun state River, with the coordinate: $7^0$89"N, $3^0$20'54"E and elevation of 66m (217ft).

Sample design and collection

Cross sectional sampling method was used in this study. The udder was cleaned thoroughly and disinfected and milk was drawn into a clean universal bottle using a gloved hand. A total of 73 samples were collected in the early hours of the day from apparently healthy lactating does from six (6) different areas that are located 2-50 kilometers apart in Abeokuta and its environs. The areas and number of samples collected are as follows: 13 samples from Directorate of University (Federal University of Agriculture), Abeokuta Farms (DUFARMS), 6 from Eweje, 6 from Odeda, 8 from Elite, 20 from Elega and 20 from Obantoko. The fresh milk samples were labeled and transported to the laboratory in an ice pack for proper preservation and were analyzed in the College of Veterinary Medicine, Department of Veterinary

Microbiology & Parasitology Laboratory.

Media / Reagent preparation

Buffered peptone water which was used as an enrichment medium was prepared by dissolving buffered peptone agar (OxoidTM, UK) into 1000mls of distilled water and then sterilized in an autoclave at a temperature of 121^{0}C for 15 minutes.

Nutrient agar preparation

Nutrient agar was prepared by suspending 28.0g of the dehydrated agar into 1000mls of distilled water, boiled to dissolve completely and the autoclaved at 121^{o}C allowed to cool. 5% sheep blood agar was made from the prepared nutrient agar with addition of 5mls of blood into every 100mls of the prepared nutrient agar after it has cooled to 40^{o}C. Slopes were also prepared this same way but at boil, were dispensed into plain bottles before it was finally autoclaved at the same temperature after which it was placed in a slanted position to solidify.

Mac Conkey agar preparation

Mac Conkey agar was prepared by suspending 52.0g of the dehydrated agar (CM 0115, OxoidTM, UK) in 1000mls of distilled water boiled to dissolve completely and sterilized by autoclaving at 121^{o}C for 15 minutes. After cooling to 50^{o}C, about 20mls was dispensed into sterile petri dishes which were left to solidify at room temperature.

Triple Sugar Iron (T.S.I) agar preparation

Triple Sugar Iron agar was prepared by suspending 66g of the dehydrated medium into 1000mls of distilled water, boiled to completely dissolve. Then the mixture was dispensed into a sterile test tube, cocked with cotton wool and sterilized by autoclaving at 121^{0}C for 15 minutes and then allowed to cool in a slanting form.

Identification of isolates

Identification was based on standard protocols (Cater, 1984). After taking note of cultural growth characteristics, positive cultures were subjected to Gram's staining to study staining properties and cellular morphology under 100 x objective of light microscope.

Mixed colonies and Gram-negative bacteria were sub-cultured on both blood and McConkey agars and incubated aerobically for further 24 hrs. Pure cultures of single colony type, from both blood and McConkey agars, were transferred onto nutrient agar-slants for Gram staining technique followed with a series of biochemical tests including, catalase, oxidase, fermentative/oxidative tests and heamolysis on blood agar for final identification, following standard procedures (Quinn *et al.*, 2002).

Antibiotics Sensitivity Test

A previously described Kirby-Bauer disc diffusion method (Masud *et al.*, 2012) was used to determine the susceptibility of the bacterial isolates against antibiotic agents. The interpretation on susceptibility was done according to the guidelines of Clinical and Laboratory Standard Institute (CLSI, 2009). Ten antimicrobial discs of antibiotics that are routinely used on the field and engaged in this antibiotic sensitivity test study include Amoxicillin, Ofloxacin, Streptomycin, Chloramphenicol, Ceftriazone, Gentamycin, Ciprofloxacin, Erythromycin, Pefloxacin and Cotrimozole. Fresh nutrient agar plate was prepared and inoculated with isolates from the old culture plates. Then the paper disc containing specific concentration of antibiotics was placed and incubated at 37^{0}C for 24hrs. The diameter of inhibition zone surrounding each antibiotic disc was measured and subsequently matched with the standard inhibition zone diameters of respective antibiotic disc. On the basis of size of inhibition zone of various antibiotics, the isolates were classified as sensitive,

intermediately sensitive or resistant (Quinn *et al.*, 2004)

Minimum Inhibition Concentration

Minimum inhibitory concentration (MIC) testing using microbroth dilution was performed on all the 182 bacteria isolates.

Statistical analysis

Programs Excel version 2003 (Microsoft ® Office Excel 2003) was used for data collection, management and analysis. Descriptive statistics were used to describe the prevalence and antibiogram was presented in percentages.

Results

The bacterial load from milk samples obtained in various locations are as follows:- DUFARMS, 0.1-1.2x10^7cfu/ml; Eweje,0.6-1.2x10^7cfu/ml; Odeda, 0.6-3.5x10^7cfu/ml;Elite,1.0-5.5x10^7cfu/ml; Elega, 0.1-2.52x10^8cfu/ml; and finally Obantoko has 0.16-1.04x10^8cfu/ml. The mean counts were 0.54 ± 0.40x10^7 cfu/ml; 0.78 ± 0.13x10^7 cfu/ml; 1.83 ± 1.23x10^7cfu/ml; 2.58 ± 1.45x10^7cfu/ml; 8.51 ± 5.60x10^7cfu/ml and 4.14 ± 3.90 x10^7cfu/ml respectively (Table 1).

Table 1: Mean Bacterial Count (x10^7cfu/ml) of Milk Sample in 6 different locations in Abeokuta

Locaton	Mean(μ)	Standard Deviation(±)
DUFARMS	0.54	0.40
Eweje	0.78	0.13
Odeda	1.83	1.23
Elite	2.58	1.45
Elega	8.51	5.60
Obantoko	4.14	3.90

Based on cultural, morphological and biochemical characteristics of organism isolated, a total of Nine (9) bacteria species comprising of 182 isolates were identified in the 73 milk samples. *Pseudomonas spp* (22.28%), *Micrococcus spp* (21.74%), *Staphylococcus aureus* (19.02%), *Staphylococcus saprophyticus* (15.22%), Enterobacter spp (8.70%) Bacillus spp (5.43%), Pasteurella spp (4.89%), *Escherichia coli* (2.17%), and *Citrobacter spp* (0.54%) were isolated. The Grams stains revealed that the isolates were predominately of Gram positive cocci (55.98%), Gram negative bacilli (39.12%) and few cocobacilli (4.89%). The prevalence of the isolates are as stated in Table 2.

Table 2: Frequency of occurrence of isolated bacteria from goat's milk in Abeokuta

Bacteria specie	Frequency of occurrence	Percentage (%)
Pseudomonas spp	41	22.28%
Micrococcus spp	40	21.74%
Staphylococcus aureus	35	19.02%
Staphylococcus saprophytcus	28	15.22%
Enterobacteria spp	16	8.70%
Bacillus spp	10	5.43%
Pasteurella spp	9	4.89%
Escherichia coli	4	2.17%
Citrobacter spp	1	0.54%

Each of the isolate was subjected to Biochemical tests for analysis.The biochemical reactions show the isolates were mostly catalase positive, Indole and coagulase negative. The reactions of the isolates to TSI agar tests are as shown in Table 3.

Table 3: Biochemical characteristics of bacteria isolated from goat's milk in Abeokuta

Bacteria	Catalase	Oxidase	Indole	Coagulase	TSI Slant/Butt	TSI H_2S	TSI Gas production
Pseudomonas spp	+ve	+ve	-ve	-ve	K/K	-ve	-ve
Micrococcus spp	+ve	+ve	-ve	-ve	K/K	-ve	-ve
Staphylococcus aureus	+ve	-ve	-ve	+ve	A/A	-ve	+ve
Staphylococcus saprophyticus	+ve	-ve	-ve	-ve	A/A	-ve	+ve
Enterobacterspp	+ve	-ve	-ve	-ve	K/A	-ve	+ve
Bacllusspp	+ve	-ve	-ve	-ve	A/K	-ve	+ve
Pasteurellaspp	+ve	+ve	-ve	-ve	K/A	-ve	+ve
Escherichia coli	+ve	-ve	+ve	-ve	A/A	-ve	+ve
Citrobacterspp	+ve	-ve	-ve	-ve	K/A	-ve	+ve

Key
K –Alkaline; A-Acidic

The 182 isolates were subjected to commonly used antibiotics and the percentage of the isolates that are sensitive or otherwise to the antibiotics were determined. While all (100%) the 182 isolates were resistant to Amoxicillin, only 11 (6.04%) were resistant to Ofloxacin. The resistance or otherwise of all the isolates to the other antibiotics used are as shown in Table 4. The response of the isolates from each bacterial species to the panel of antibiotics was determined and the result is presented in Table 5 while the MIC results of each antibiotic to the isolates are also shown in Table 6.

Table 4: Antibiogram of bacteria isolated from goats milk in Abeokuta

ANTIBIOTICS	SENSITIVE	INTERMEDIATE	RESISTANT
Amoxicillin	-	-	182(100%)
Ofloxacin	161(88.50%)	10(5.49%)	11(6.04%)
Streptomycin	11(6.04%)	17(9.34%)	154(84.62%)
Chloramphenicol	5(2.75%)	27(14.84%)	150(82.42%)
Ceftriazone	-	25(13.74%)	157(86.26%)
Gentamycin	20(10.99%)	26(14.29%)	136(74.73%)
Ciprofloxacin	122(67.03%)	29(15.93%)	31(17.03%)
Erythromycin	2(1.09%)	43(23.63%)	137(75.27%)
Pefloxacin	149(81.87%)	12(6.59%)	21(11.54%)
Cotrimoxazole	25(13.74%)	15(8.24%)	142(78.02%)

Amoxicillin(25µg),Ofloxacin(5µg), Streptomycin(10µg), Chloramphenicol(30µg), Ceftriazone(30µg), Gentamycin(10µg), Ciprofloxacin(10µg), Erythromycin(5µg), Pefloxacin (5µg), Cotrimoxazole (25µg). S= Susceptible; I = Intermediate; R = Resistant.

Table 5: Antimicrobial susceptibility of isolates from milk samples

ISOLATES	AMX			OFX			STR			CHL			CFZ			GN			CPX			ERY			PEF	COT				
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Pseudo (23)	1	1	21	16	1	6	3	7	13	1	4	18	4	2	17	3	2	18	10	4	9	15	2	6	12	3	8	3	4	16
Micro (20)	3	2	15	14	2	4	4	9	7	2	5	13	3	1	16	2	1	17	9	5	6	4	0	16	7	1	12	4	6	10
Stapha (12)	0	2	10	10	2	0	2	4	6	3	7	2	2	4	6	2	3	7	4	3	5	3	1	8	5	2	5	2	3	7
Staphpp (14)	1	4	9	12	2	0	4	1	9	3	6	5	3	3	8	22	4	8	5	4	5	4	1	10	10	2	2	2	7	5
Bac (3)	0	1	2	3	0	0	1	1	1	0	3	0	1	2	0	1	1	1	2	1	0	2	0	1	2	1	1	2	1	0
Past (4)	0	1	3	4	0	0	2	1	1	2	1	1	1	0	3	0	1	3	2	0	2	2	0	2	2	0	2	2	0	2
Ec (4)	0	0	4	4	0	0	0	2	2	2	1	1	1	0	3	0	2	2	2	1	1	1	1	2	2	0	2	2	1	1
Citr (1)	0	1	0	1	0	0	0	1	0	1	0	0	1	0	0	1	1	0	0	1	0	0	1	0	1	0	0	1	0	0

KEY:
Amx=Amoxycillin; Cfz=ceftazidine; Pef=pefloxacin; Ofx=ofloxacin., Gn=gentamycin, Cot=cotrimoxazole; Str=streptomycin; Cpx=ciprofloxacin; Chl=chloramphenicol; Ery=erythromycin. Pseudo=*Pseudomonas aeruginosa* ; Micro=*Micrococcus* species; Stapha=*Staphylococcus aureus*; Staphpp=*Staphy.saprophyticus*; Bac=*Bacillus subtilis*; Past=*Pasteurella*species;*Ec=Escherichia coli; Citr=Citrobacter*species

Table 6: Minimum inhibitiory concetration of isolates from milk sample showing mic ≥8 ug/ml

	Amx		Ofx		Str		Chl		Cfz		Gn		Cpx		Ery		Pef		Cot	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Pseudo (23)	1	22	16	7	5	18	10	13	4	19	14	9	16	7	14	9	18	5	4	19
Micro (20)	0	20	10	10	7	13	6	14	3	17	10	10	12	8	12	8	14	6	2	18
Stapha (12)	0	12	6	6	6	6	4	8	2	10	6	6	6	6	4	8	12	0	2	10
Staphpp(14)	2	12	8	6	5	9	2	12	2	12	3	11	2	12	3	11	2	12	2	12
Bac (3)	3	0	2	1	2	1	1	2	1	2	2	1	0	3	2	1	1	2	0	3
Past (4)	4	0	3	1	2	2	0	4	2	2	0	4	1	3	0	4	1	3	0	4
Ec (4)	2	2	2	2	0	4	0	4	0	4	1	3	0	4	0	4	1	3	0	4
Citr (1)	0	1	1	0	0	1	0	1	0	1	1	0	0	1	0	1	1	0	0	1

NOTE: MIC ≥8 mg/ml are referred to as susceptible while greater than 8ug/ml were resistant strains

Discussions

Milk is synthesized in specialized cells of the mammary gland and is virtually sterile when secreted into the alveoli of the udder (Tolle, 1980). Beyond this stage of milk production, microbial contamination can generally occur from three main sources viz- from within the udder during clinical and sub-clinical infections, from the exterior of the udder, and from the surface of milk handling and storage equipment (Bramley and McKinnon, 1990). Raw milk as it leaves the udder of healthy goats normally contains very low numbers of microorganisms. Mean counts in goats for total bacterial, coliform and staphylococcus were 2.54×10^4., 0.966×10^3., 3.32×10^3 /ml respectively (Park and Humphrey (1986).The mean count data collected in this study is higherapparently because of the management system. In DURAFARMS, the semi-intensive is practised and its mean bacterial cell count at 0.54×10^7cfu/ml is lower than the mean counts for all other areas that practice range management system. The microbes isolated in this study also compare well with the works of others (Rhamsahoi *et al.*, 2011; Kyozaire *et al.*, 2005). The prevalence of each organism isolated is higher in this studypossibly because of the management system.

The goats in this study are presumed to be healthy. While a healthy udder should contribute very little to the total bacteria count inraw milk, a dairy animal presumed to be healthy may have sub-clinical mastitis and has the potential to shed large numbers of microorganisms into the raw milk supply (Bramley and McKinnon, 1990). This might partly explain the relatively high bacterial milk count recorded in presumably healthy goats in this study. While mastitis organisms commonly found to influence total raw milk count are *Streptococcus species*, most notably *S. agalactiae* and *S. uberis* (Bramley and McKinnon, 1990; Bramley *et al*., 1984; Jeffrey and Wilson, 1987), there are others such as *Pseudomonas*, *Micrococcus* and *Pasteurella* species which also have the potential to contaminate fresh milk (Bramley and McKinnon 1990). However detection of these implied pathogens does not necessarily indicate that they originated from the milk of dairy animals. Potential environmental mastitis pathogens and/or similar organisms can occur in raw milk as a result of other contributing factors such as dirty environment, poor cleaning, and or dirty hands and materials/equipment for collecting the milk (Gonzalez *et al.,* 1986). Milking heavily soiled dairy animals could potentially result in raw milk counts exceeding 10^4 per ml. Several studies have

investigated pre-milking udder hygiene techniques in relation to the bacteria count of milk (Bramley and McKinnon, 1990; Galton *et al.*, 1984; Pankey 1989; McKinnon *et al*., 1990). These organisms are by nature associated with the dairy animals' environment and may influence raw milk bacteria counts through other means (Bramley, 1982; Zehner *et al*., 1986). *Staphylococcus aureus* is not thought to be a frequent contributor to total counts although a mean count of 60,000/ml has been documented (Gonzalez *et al.,* 1986). Therefore the *Staphylococcus aureus* count of 35 x 10^7/ml recorded in this study may not be too high when the poor environmental hygiene such as soiled teats, barnyard mud and other bad management factors listed above are considered as key factors influencing the level of microbial contamination of raw milk. The role of these key environmental factors would require further studies. The antibiotic sensitivity pattern of the isolates in this study was presented in Table 4. Of the 10 antimicrobial discs of antibiotics routinely used on the field, Amoxicillin, a derivative of penicillin, is the least effective because all the 182 bacterial isolates (100%) were resistant to it. The results are consistent with the studies of Begum *et al.* (2012) and Da Silva *et al*. (2004) that bacteria in goat mastitis are more resistant to Penicillin G. Similarly in this study, 86.26% of the isolates are resistant to Ceftriazone, a third generation Cephalosporin, that works like penicillin. The resistance of the isolates is also high to Streptomycin (84.62%) and its other derivatives erythromycin, 75.27%, and Gentamycin, 74.73%. Since Penicillin and Streptomycin have been in the market for a long time and used extensively along with their derivatives for treatment of infections, including mastitis in livestock, this extensive use or overuse may have led to the development of high resistance of the bacteria against this first line generation of antibiotics (Tras *et al.,* 2007). The isolates are equally resistant to aminoglycosides and derivatives such as Streptomycin (84.62%), erythromycins, 75.27% and Gentamycins 74.73%. These also are first-line antibiotics that have been subjected to indiscriminate and/or overuse, as Penicillin. However it is evident from Table 4, that all the isolates are susceptible to Oxaflocin with only 6.04% resistant isolates and resistant to Pefloxacin with only 11.54% resistant isolates and Ciproflocaxin (17.0%). These antibiotics are derivatives of second generation Quinolones with group specific activities against the aerobic gram-negative bacilli (Oliphant and Green, 2002). They have exceptionally high intra-cellular concentrations, are relatively new and therefore less abused compared to the Penicillin derivatives. A good number of the isolates in Table 4 are susceptible to these antibiotics. These antibiotics therefore are suggested options for treatment of infections in goats in this study.

In this study, the use of MIC was done to determine precisely the concentration of the antibiotic required to inhibit growth of each isolate (CLSI, 2013) and most of the isolates have shown resistance to Amocillin, Cotrimazole, Choramphenicol and Ceftazidine. These antibiotics, like many others, in Nigeria and developing countries, are cheap and easily accessible and are easily bought off the shelf without appropriate prescription by practicing veterinarians. The surveillance of resistance in many developing countries is suboptimal. The general picture is one of accelerating rates of resistance spurred by antimicrobial misuse and shortfalls in infection control. This may have led to an increased resistance that has been reported by several authors (Morrissey *et al.,* 2013).

Recommendations

Consumption of dairy milk products from goats is on the increase in Nigeria therefore further work needs to be done to assess the level of pathogenic organisms in milk from goats presumed to be healthy. There is the need to clarify the role of environmental factors, agents and transmission of bacterial resistance as well as standardize sampling and laboratory procedures. Reservoirs for resistance may be present in healthy animal populations and these reservoirs need to be investigated to understand means of transfer of bacterial resistance. Considerable economic and health burdens emanate from bacterial resistance, and research is needed to accurately quantify the problem, propose and evaluate practicable solutions.

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