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Incidence and antibiotic susceptibility of bacteria in goat milk in Ibadan, Nigeria

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Abstract

Goat milk and its products are highly nutritious and widely consumed in several countries. In Nigeria, little attention has been given to goats as dairy animals; hence the raw milk can be a potential source of bacterial contamination and spread of antibiotic resistant pathogens within human, animal and environment. In this study, the incidence and antibiotic susceptibility of bacteria in goat milk in Ibadan, Nigeria was evaluated. The bacteriological quality and antibiotic resistance characteristics of the isolates from105 raw milk samples collected from does in five goat herds in Ibadan in Oyo-State, Nigeria were carried out. A total of 126 bacterial isolates belonging to six bacteria genera [Lactobacillus spp (23.81%), Staphylococcus aureus (23.02%), E.coli (20.63%), Klebsiella pneumonia (15.08%), Pseudomonas aeruginosa (10.35%), Streptococcus spp (07.14%)] were obtained from the samples. The Gram negative isolates exhibited resistance to amoxillin (84.50%), augmentin (77.59%), septrin (75.86%), sparfloxacin (72.41%), chloramphenicol (68.97%), streptomycin (65.52%), perfloxacin (56.90%), tarivid (51.72%), gentamycin (48.28%), ciprofloxacin (48.27%).While the Gram positive bacteria also exhibited resistance to ampiclox (69.12%), septrin (42.65%), erythromycin (33.82%), streptomycin (14.71%), gentamycin (11.76%), rocephin (07.35%), zinnacef (02.94%), ciprofloxacin (01.47%). Multi-drug resistance (MDR) to three or more antimicrobials was observed in some of the isolates.This study revealed high prevalence of MDR bacteria in goat milk that can contribute to the global antibiotic resistance menace through primary or secondary infections associated with husbandry, milking and consumption of improperly pasteurized goat milk.

Keywords: Incidence, Antibiotic Susceptibility, Bacteria, Goat Milk, Ibadan, Nigeria.

Introduction

Goat herding is an important aspect of the nutritional economy for many developing countries, supporting both the meat and dairy industry. Goat milk provides excellent nutritional supplements with high quality protein for infants and adults. It also provides essential nutrients for body maintenance in adults and a good source of calcium supplements for both expectant and lactation mothers (Verraes, 2015). Other unique and beneficial characteristics of goat milk such as improved digestibility, low lactose content, higher buffering capacity and specific therapeutic value make it superior to bovine milk (Fillimon *et al.,* 2011). Relative to cow milk which is acidic, goat milk is distinctly alkaline, thereby making ituseful in the treatment of hyperacidity (Park *et al.,* 2007). Another feature of goat milk is the rareness of tubercle bacillus, although it can occur. Goat milk has, thereby making it good for lactose intolerant people (Almon *et al.*, 2007). The unique constituents of goat milk and the contributions to diet and livelihood

in developing countries require hygiene and quality assessment. Food safety consideration and udder health management are critical to producing hygienic milk and milk products. Therefore, intramammary infections are of great importance to milk hygiene in dairy goat (Moroni *et al.,* 2005). The milk can get contaminated by various pathogenic or spoilage microorganisms (mainly bacteria) during milking processes and storage from farm upto table. The presence of high microbial load in milk can pose major economic loss for local farmers and small scaledairies (Metz *et al.*, 2009; Suguna *et al.*, 2012). Consumption of raw milk could pose risk for the consumers due topossible presence ofpathogenic microorganisms in the raw milk (Claeys *et al.,* 2013). The pathogens that could be present in raw milk could be endogenous or from environmental contamination environment during the collection or storage of the milk. The types of bacteria present in milk can influence the milk products, longer shelf-life which is attributed to high proportion of short and medium chain fatty acids and can promote health or cause disease in consumers of the milk and milk products (FAO, 1997). Microbial profile of raw milk can also provide insight into the health status of the lactating dam since it changes during the course of lactation (D'Amico and Donnelly, 2010) and in response to infections such as mastitis (Alawa *et al.*, 2000). Some beneficial bacteria found in milk (such as *Lactobacillus spp or Bifidobacterium spp)* can be present in human gastrointestinal tract as commensals and aiding digestion. Pathogenic bacteria including zoonotic organisms are also detected in raw milk and other dairy products. *Brucella abortus, B. melitensis, Campylobacter jejuni, Escherchia coli, Listeria monocytogense, Mycobacterium bovis, M. tuberculosis, Salmonella, Staphylococcus aureus* and *Yersinia enterocolitica* have been implicated as zoonotic milk borne pathogens (Pal, 2012a); (FAO, 2013). Microbial contamination of milk during the milking processes could be inevitable depending on the husbandry and hygiene practices. Therefore, the microbial content of milk is critical to the quality and safety of the milk (Singh *et al.*, 2011). In Nigeria, little attention has been given to goats as dairy animals; hence their raw milk could be a potential source of bacterial contamination and spread of antibiotic resistant pathogens within human, animal and environment.The aim of this study was to investigate the occurrence of bacteria isolates that can be found in raw goat milk in Ibadan and to determine their resistance to various antibiotics.

Materials and methods

Samples and sampling procedures

A total of 105 raw milk samples were collected randomlyfrom West African dwarf and red Sokoto does. Freshly expressed raw milk samples were collected from apparently healthy lactating does in 5 herds located in Bodija areas of Ibadan, Oyo-State, Nigeria. Five milliliters of raw milk were collected directly from each of the lactating doe into sterile universal bottles after cleaning the udder with warm disinfectant solution. One sample was obtained from an individual doe on each visit. Samples were properly labeled and transported in cooler with ice-packs to laboratory for immediate microbiological analysis.

Isolation and identification of organism

One millilitre of each fresh milk samples was inoculated into 9 ml of sterile tryptic soy broth (TSB, Oxoid®, Basingstoke, UK) in a universal bottle for pre-enrichment. The pre-enrichment culture was incubated at 37 °C for 8 hours.

Following pre-enrichment, aloopful of the TSB culture was inoculated onto blood agar and MacConkey agar plates, the plates were incubated at 37 °C for 18 - 24 hours.After incubation, morphological characterization of each colony was carried out. Subsequently, the colonies were stained using Gram's Methodtechnique and examined microscopically. Biochemical test were performed according to the method described by Cowan and Steel's laboratory manual for the characterization of each isolate: catalase reaction, oxidase test, coagulase test was carried out for Gram positive cocci in clusters, urease test, citrate utilization test, indole test, motility test and carbohydrate fermentation test for the following sugars: sucrose, glucose, salicin, mannitol, lactose and dulcitol. Results of biochemical tests were interpreted using Cowan and Steel's manual for the identification of medical bacteria (Barrow *et al.,* 2005).

Antimicrobial susceptibility test

The susceptibility of identifiedisolates to antimicrobial agents was determined by the standard Kirby-Bauer disk diffusion method. A single colony of the isolate under test was inoculated unto TSB and incubated for 8- 12 h. After incubation, the turbidity of the TSB culture was adjusted to 0.5 McFarland standards. A sterile swab was dipped into the adjusted TSB culture and inoculated onto Mueller-Hinton agar (MHA) (Oxoid, Basingstoke, UK) plate by swabbing the entire surface of the MHA with the adjusted TSB culture. The antimicrobial disks were individually placed firm on the inoculated MHA plate. The plates were incubated at 37°C for 18 - 24 h. After incubation, the diameter of the clear zone of inhibition around each antimicrobial disk was measured (in millimeters) and the result was interpreted in accordance with the recommendation of Clinical and Laboratory Standards Institute (CLSI), (2008). Susceptibility and resistance to the following antimicrobials was determined for the 58 Gram negative isolates: amoxicillin (25µg), perfloxacin (10µg), chloramphenicol (30µg), ciprofloxacin (5µg), sparfloxacin (10µg), septrin (30µg), gentamicin (10 µg), augmentin (30µg), streptomycin (10µg) and ofloxacin (5µg). Also, susceptibility and resistance to the sixty eight Gram positive isolates was determined for the following antimicrobials: pefloxacin (10µg), ampiclox (10µg), gentamycin (10µg), zinnacef (20µg), amoxacillin (25µg), rocephin (25µg), ciprofloxacin (5µg), streptomycin (10µg), septrin (30µg) and erythromycin (10µg).

Results

A total of 126 bacterial isolates were obtained from 105 raw milk samples of healthy does. *Lactobacillus spp* (23.81%), followed by *Staphylococcus aureus* (23.02%), *E. coli (*20.63%), *Klebsiella pneumonia* (15.08%), *Pseudomonas aeruginosa* (10.35%), *Streptococcus spp* (07.14%), (Table 1). *Lactobacillus spp* and *Escherichia coli* ranked highest in the isolation frequency for Gram positive and Gram negative bacteria, respectively (Table 1).

Table 1: Distribution of bacteria isolates

Bacteria isolates	Frequency (%)
Gram positive bacteria	**68 (53.97)**
Lactobacillus spp	30 (23.81)
Staphylococcus aureus	29 (23.02)
Streptococcus species	09 (07.14)
Gram negative bacteria	**58 (46.03)**
Escherichia coli	26 (20.63)
Klebsiella species	19 (15.08)
Total number of isolates	**126 (100.0%)**

The overall rate of *Lactobacillus spp* (n=30) had 13(43.3%), 10(33.3%), 06(20.0%), 05(16.7%), 4(13.3), 04(13.3%), 3(10%), 01(3.3%), 0(0%) and 0(0%) resistance to ampiclox, erythromycin, perfloxacin, septrin, amoxicillin, gentamycin, streptomycin, rocephin, ciprofloxacin and zinnacef, respectively. *Staphylococcus aureus* (n= 29) displayed 27(93.1%), 19(65.5%), 10(34.5%), 09(31.0%), 04(13.8%), 04(13.8%), 03(10.3%), 02(6.9%), 01(3.4%) and 0(0%) resistance to ampiclox, septrin, erythromycin, amoxicillin, perfloxacin, streptomycin, gentamycin, rocephin, zinnacef, and ciprofloxacin, respectively. *Streptococcus species* (n=09) showed 07 (77.8%), 05 (55.6%), 05 (55.6%), 03 (33.3%), 03 (33.3%), 03 (33.3), 02 (22.2%), 01 (11.1%), 01 (11.1%) and 01 (11.1%) resistance to ampiclox, amoxicillin, septrin, perfloxacin, streptomycin, erythromycin, rocephin, gentamycin, zinnacef, and ciprofloxacin, respectively (Table 2).

Table 2: Antibiotic susceptibility pattern for Gram positive bacteria

Antibiotic	Organism	*Lactobacillus spp* n = 30	*Staphylococcus aureus* n = 29	*Streptococcus species* n = 09	Total n=68
Ciprofloxacin	Sensitivity	30 (100)	29 (100)	08 (88.9)	67 (98.53)
	Resistance	0(0)	0 (0)	01(11.1)	01 (1.47)
Perfloxacin	Sensitivity	24 (80.0)	25(86.2)	06 (66.7)	55(80.88)
	Resistance	06(20.0)	04 (13.8%)	03(33.3)	13 (19.12)
Rocephin	Sensitivity	29 (96.7)	27(93.1)	07(77.8)	63 (92.65)
	Resistance	01(3.3)	02(6.9)	02(22.2)	05 (7.35)
Gentamycin	Sensitivity	26 (86.7)	26(89.7)	08 (88.9)	60 (88.24)
	Resistance	04(13.3)	03(10.3)	01(11.1)	08 (11.76)
Zinnacef	Sensitivity	30 (100)	28 (96.6)	08 (88.9)	66 (97.06)
	Resistance	0(0)	01(3.4)	01(11.1)	02 (2.94)
Erythromycin	Sensitivity	20 (66.7)	19 (65.5)	06 (66.7)	45 (66.18)
	Resistance	10(33.3)	10(34.5)	03(33.3)	23 (33.82)
Streptomycin	Sensitivity	27 (90.0)	25 (86.2)	06 (66.7)	58 (85.29)
	Resistance	03(10.0)	04(13.8)	03(33.3)	10 (14.71)
Septrin	Sensitivity	25 (83.3)	10 (34.5)	04 (44.4)	39 (57.35)
	Resistance	05(16.7)	19(65.5)	05(55.6)	29 (42.65)
Amoxacillin	Sensitivity	26 (86.7)	20(69.0)	04 (44.4)	50 (73.53)
	Resistance	04(13.3)	09(31.0)	05(55.6)	18 (26.47)
Ampiclox	Sensitivity	17 (56.7)	02(6.9)	02 (22.2)	21 (30.88)
	Resistance	13(43.3)	27(93.1)	07(77.8)	47 (69.12)

Escherichia coli (n= 26) displayed 25 (96.5%), 25 (96.5%), 24 (92.3%), 23 (88.5%), 22 (84.2%), 21 (80.4%), 19 (73.1%), 17 (65.4%), 17 (65.4%) and 11 (42.3%) to augmentin, septrin, sparfloxacin, chloramphenicol, amoxacillin, streptomycin, perfloxacin, ofloxacin, ciprofloxacin and gentamycin, respectively. *Klebsiella species (n=19)* showed 09 (47.4%), 08 (42.1%), 08 (42.1%), 08 (42.1%), 08 (42.1%), 08 (42.1%), 7 (36.8%), 7 (36.8%), 6 (31.6%) and 5 (26.3%) resistance to amoxacillin, septrin, augmentin, sparfloxacin, streptomycin, perfloxacin, chloramphenicol, ofloxacin, ciprofloxacin and gentamycin, respectively. *Pseudomonas species* (n=13) showed 12 (92.3%), 11 (84.6%), 11 (84.6%), 10 (76.9%), 10 (76.9%), 9 (69.2%), 6 (46.2%), 6 (46.2%), 5 (38.5%) and 3 (23.1%) resistance to augmentin, amoxacillin, septrin, chloramphenicol, sparfloxacin, streptomycin, perfloxacin, ofloxacin ciprofloxacin and gentamycin, respectively (Table 3).

Table 3: Antibiotic susceptibility patterns for Gram positive isolates

Test drugs	Amount (µg)	Sensitive (%)	Resistant (%)
Pefloxacin	10	55 (80.88)	13(19.12)
Gentamycin	10	60(11.76)	8(11.76)
Ampiclox	10	21(30.88)	47(69.12)
Zinnacef	20	66(97.06)	2(02.94)
Amoxicillin	25	50(73.53)	18(24.47)
Rocephin	25	63(92.65)	5(07.35)
Ciprofloxacin	5	67(98.53)	1(01.47)
Streptomycin	10	58(85.29)	10(14.71)
Septrin	30	39(57.35)	29(42.65)
Erythromycin	10	45(66.18)	23(33.82)

Table 4: Percentage distribution of antibiotic susceptibility of Gram positive bacteria isolates

Antibiotics.	Staph. aureus (n=29)	Streptococcus Spp. (n=9)	Lactobacillus spp. (n=30)	Total Resistance
Pefloxacin	04(13.8)	03(33.3)	06(20.0)	13(19.12)
Gentamycin	03(10.3)	01(11.1)	04(13.3)	8(11.76)
Ampiclox	27(93.1)	07(77.8)	13(43.3)	47(69.12)
Zinnacef	01(3.4)	01(11.1)	0(0)	2(02.94)
Amoxacillin	09(31.0)	05(55.6)	04(13.3)	18(24.47)
Rocephin	02(6.9)	02(22.2)	01(3.3)	5(07.35)
Ciprofloxacin	0 (0)	01(11.1)	0(0)	1(01.47)
Streptomycin	04(13.8)	03(33.3)	03(10.0)	10(14.71)
Septrin	19(65.5)	05(55.6)	05(16.7)	29(42.65)
Erythromycin	10(34.5)	03(33.3)	10(33.3)	23(33.82)

Table 5: Antibiotic susceptibility pattern for Gram negative bacteria

Antibiotic		Organism			Total n=58
		E. coli n = 26	*Klebsiella spp* n = 19	*Pseudomonas spp* n = 13	
Ciprofloxacin	Sensitivity	09(34.6)	13(68.42)	08(61.54)	30(51.72)
	Resistance	17(65.4)	06(31.58)	05(38.46)	28(48.28)
Pefloxacin	Sensitivity	07(26.92)	11(57.89)	07(53.85)	25(43.10)
	Resistance	19(73.08)	08(42.11)	06(46.15)	33(56.90)
Chloramphenicol	Sensitivity	03(11.54)	12(63.16)	03(23.08)	18(31.03)
	Resistance	23(88.46)	07(36.84)	10(76.92)	40(68.97)
Gentamycin	Sensitivity	15 (57.69)	14(73.68)	16(84.21)	39(67.24)
	Resistance	11(42.31)	05(26.32)	03(15.79)	19(32.76)
Sparfloxacin	Sensitivity	02(07.69)	11(57.89)	03(23.08)	16(27.59)
	Resistance	24(92.31)	08(42.11)	10(76.92)	42(72.41)
Augmentin	Sensitivity	01(03.85)	11(57.89)	01(07.69)	13(22.14)
	Resistance	25(96.15)	08(42.11)	12(92.31)	45(77.59)
Streptomycin	Sensitivity	05(19.23)	11(57.89)	04(30.77)	20(34.48)
	Resistance	21(80.77)	08(42.11)	09(69.23)	38(65.52)
Septrin	Sensitivity	01(03.85)	11(57.89)	02(15.38)	14(24.14)
	Resistance	25(96.15)	08(42.11)	11(84.62)	44(75.86)
Amoxacillin	Sensitivity	04(15.38)	10(52.63)	02(15.38)	16(27.59)
	Resistance	22(84.62)	09(47.37)	11(84.62)	42(72.41)
Ofloxacin	Sensitivity	09(34.62)	12(63.16)	07(53.85)	28(48.28)
	Resistance	17(65.38)	07(36.84)	06(46.15)	30(51.72)

Table 6: Antibiotic susceptibility pattern for Gram negative bacteria

Antibiotics	*Escherichia coli* (26)	*Klebsiella pneumonia* (19)	*Pseudomonas aeruginosa* (13)	Total Resistance
Septrin	25(96.2)	08 (42.1)	11(84.6)	44(75.86)
Chloramphenicol	23(88.5)	07(36.8)	10(76.9)	40(68.97)
Sparfloxacin	24(92.3)	08(42.1)	10(76.9)	42(72.41)
Ciprofloxacin	17(65.4)	06(31.6)	05(38.5)	28(48.27)
Amoxacillin	22(84.2)	09(47.4)	11(84.6)	42(84.50)
Augmentin	25(96.2)	08(42.1)	12(92.3)	45(77.59)
Gentamycin	11(42.3)	05(26.3)	03(23.1)	19(48.28)
Pefloxacin	19(73.1)	08(42.1)	06(46.2)	33(56.90)
Ofloxacin	17(65.4)	07(36.8)	06(46.2)	30(51.72)
Streptomycin	21(80.4)	08(42.1)	09(69.2)	38(65.52)

Discussion

The milk of small ruminant plays an important role in the nutrition of both agricultural and urban areas. Goat's milk can be laddened with endogenous and environmental pathogenic and spoilage microbial contaminants that can pose serious health risks to the consumers especially to infants, elderly and immune-compromised individual. Dairy development efforts in Nigeria are beginning to incorporate goat milk to boost local production and consumption of milk.

In this study, we isolated *Lactobacillus spp, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, S. aureus and Streptococcus species* from raw milk of lactating does from five herds located in Bodija areas of Ibadan, Oyo- State, Nigeria. Out of the overall 126 bacterial isolated, thirty (23.81%) were identified as *Lactobacillus spp,* i.e. a common beneficial bacteria in milk. While the *Staphylococcus aureus* (23.02%), *Escherichia coli.* (20.63%) *Klebsiella pneumoniae* (7.14%) *Pseudomonas aeruginosa* (15.08%), *Streptococcus species* (10.35%) are pathogenic. The level of contamination obtained in this study similar to the results of survey of raw milk conducted in other countries (Coeuret *et al.*, 2003; Zouharova and Rysanek, 2008; Ertas *et al.,* 2010; Mørk *et al.*, 2010; Tang *et al.,* 2011). *Lactobacillus species* and *Escherichia coli* ranked highest for Gram positive and Gram negative bacteria, respectively. This is in agreement with Tambekar and Bhutada (2010) who reported high prevalence of *these two bacteria in goat raw milk. Although the Lactobacillus species belongs to Lactic acid producing bacteria (LAPB) which are known to* improve food quality and also play an important role in preventing the growth of undesirable bacteria. The *Lactobacillus spp.* can produce some metabolites such as Bacteriocins like Acidophilin, Acidolin, Lactocidin, Bulgarican, Lactolin, Lactobacillin and Lactobrevin which are antagonistic to various degrees against diarrheagenic intestinal pathogens (Tambekar and Bhutada, 2010). *Lactobacillus* spp producing bacteriocin could be very useful in controlling the serious food borne contaminants entering into goat by various means. *Lactobacillus* sp also produce antibacterial compound could also save the consumer from pathogenic microbes when they drink goat milk and eat value added products produced from goat milk. All the isolates exhibited MDR against commonly used antibiotics with variable resistance patterns against one or more antimicrobial agent. The high MDR in the isolates obtained in this study is of great concern and indicate the role of goats in the epidemiology of antibiotic resistance. Antibiotics are used to treat diseases of cattle, sheep, goats, water buffalo, plants and other animals as

well as preservatives for milk (Devriese *et al.*, 1997). The indiscriminate use of antibiotics has led to the development of multiple antibiotic resistances thereby rendering the antibiotic treatment ineffective transmissible within soil, water, plant and animals. It has been estimated that nearly equal tonnage of antimicrobial agents are used in man and in agriculture worldwide (Farzana *et al.*, 2004). Antimicrobial resistance is a major public health concern in many countries due to the persistent circulation of resistant strains of bacteria in the environment and the possible contamination of water and food (Normanno *et al.*, 2007). *S. aureus* has been reported to frequently show multiple antimicrobial resistance patterns (Enright, 2003). Therefore, there should be effective goat health management and hygienic milking of goats. Adequate pasteurization is also recommended to reduce bacterial contamination while consumption of raw milk should be discouraged.

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