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Research Article

Vaginal Bacterial Isolation and Antimicrobial Susceptibility Patterns from Non-Pregnant Sahel Goats

Jashilagari Stephen[1], Abubakar Sadiq Lawan[1], Stephen Bitrus Balami[2], Ismail Saleh Musa[1], *Mohammed Ali[3], Innocent Damudu Peter[1] and Victoria Adam Maina[1]

[1]Department of Theriogenology, Faculty of Veterinary Medicine, University of Maiduguri, 600230 Maiduguri, Nigeria
[2]Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Maiduguri, 600230 Maiduguri, Nigeria
[3]Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Maiduguri, 600230 Maiduguri, Nigeria
***Corresponding Author's email*: vetmadeali@gmail.com; Phone: +23408032097911**

ABSTRACT

Antimicrobial resistance (AMR) poses a significant global health challenge. The emergence of AMR is particularly disturbing as it can have far-reaching consequences for animal health, human health, and the environment. This study investigated the occurrence of vaginal bacteria and their antimicrobial susceptibility patterns amongst non-pregnant Sahel goats. Sahel goats are a distinct population found in the Sahel region of Africa. Vaginal samples from 25 goats were examined. Fifteen (15) out of 25 samples (60%) had bacterial growth, which yielded Sixty-four (64) bacterial isolates. The isolates comprised 35 Gram-positive bacteria (54.7%) and 29 Gram-negative bacteria (45.3%). Antimicrobial susceptibility testing revealed alarming resistance among Gram-negative bacteria, particularly *E.coli*, which exhibited 68.8% resistance to Nitrofurantoin, 75% resistance to Ceftriaxone, and 81.3% resistance to Oxacillin. Conversely, Gram-positive bacteria, primarily *Staphylococcus aureus*, showed high susceptibility to Vancomycin (80%). The findings of this study underscore the urgent need for action to mitigate the rise of AMR in veterinary settings. Implementing judicious antibiotic use and targeted interventions is critical to safeguarding animal health, protecting human health, and preserving the environment.

Keywords: Antibiotics; Bacteria; Sahel goat; Susceptibility; Vagina

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INTRODUCTION

Goats (*Capra hircus*) are among the earliest domesticated animals, with archaeological evidence suggesting initial domestication in the Fertile Crescent around 10,000 years ago (Zeder and Hesse, 2000). Molecular research by Naderi *et al*. (2008) indicates that goats originated from bezoars (*Capra aegagrus*) in West Asia. Following their domestication, goats played a pivotal role in the Neolithic agricultural revolution and the advancement of human civilization, spreading globally to inhabit diverse environments. Today, approximately 840 million goats are found on every continent except Antarctica, providing vital sources of meat, milk and fiber (FAO, STAT, 2013).

In Nigeria, goats play a vital role in farming activities, particularly for smallholder farmers (Akpa *et al*., 2000). However, genital infections caused by opportunistic bacteria like *Escherichia coli*, hinder reproductive efficiency (El-Arabi *et al*., 2013).

The microbiota, comprising microorganisms and viruses, inhabits animal epithelia, creating a complex ecosystem (Pascale *et al*., 2018). Prokaryotes, mainly bacteria, live in symbiosis with the host. However, environmental and immune

system imbalances can lead to negative health effects, making some microorganisms pathogenic (Maynard *et al*., 2012; Belkaid *et al*., 2013).

Ruminal microbiota plays a crucial role in cellulose digestion, providing energy for ruminants (Henderson *et al*., 2015). Recent studies highlight the importance of the vaginal microbiota (VM) in ruminants, varying according to physiological and reproductive states, estrous cycle and breed (Manes *et al*., 2018; Giannattasio-Ferraz *et al*., 2019).

Reproductive efficiency is essential in sheep and goat production, as diseases can cause infertility or reduce productivity (El-Arabi *et al*., 2013). Genital infections, often caused by opportunistic secondary invaders like *Escherichia coli*, can lead to reproductive failure in ruminants (Sargison *et al*., 2007; Manes *et al*., 2010).

Understanding the composition of the VM and their antimicrobial susceptibility in goats is crucial for identifying opportunistic bacterial populations that could trigger clinical and subclinical vaginitis, ultimately improving reproductive efficiency and addressing production problems.

MATERIALS AND METHODS

Study Area

The study was conducted at Maiduguri Central Abattoir, situated in Maiduguri, the capital city of Borno State. The abattoir is geographically located between longitude 013.10719°E and latitude 11.51519°N. Maiduguri city lies at an elevation of 300 meters above sea level, within the coordinates 11.46°N - 11.54°N and 13.04°E - 13.14°E. With a population of approximately 844,747 residents and covering an area of 72,609 square kilometers, Maiduguri is the largest city in Borno State **(**Abatcha *et al*., 2024).

Collection of Samples

A total of 25 vaginal swab samples were aseptically collected from goats at the Maiduguri Central Abattoir, before slaughter. Sample collection was performed using disposable sterile swabs (Greiner) with transport media. To minimize contamination, the outer surface of the vagina was disinfected with 70% ethanol before gently inserting the swabs into the vaginal cavity to collect the samples.

Isolation and Identification of Bacteria

The isolation and identification of bacteria from the collected samples were performed using a combination of cultural, biochemical, and physiological characterization techniques. The samples were inoculated onto a range of selective and differential media, including blood agar, MacConkey agar, Mannitol Salt agar, Eosin Methylene Blue agar, Nutrient agar, Tetrathionate broth, and Salmonella Shigella agar, and incubated at 37°C for 24-48 hours to facilitate bacterial growth. Biochemical characterization of the isolates was then performed using a panel of tests, including catalase, coagulase, indole, methyl Red, Voges-Proskauer, Citrate utilisation (IMVIC), Triple Sugar Iron (TSI), and motility tests, which enabled the determination of the bacterial isolates' identities and characteristics, interpreted according to established protocols (Quinn *et al.*, 2004).

Antibacterial susceptibility testing

Antibiotic susceptibility testing of the bacterial isolates was conducted using the Kirby-Bauer disk diffusion method on Muller- Hinton agar (HIMEDIA, Mumbai), following the protocols established by the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2016). Ten (10) different antibiotic discs were selected to be tested against each bacterial isolate. Bacterial colonies from pure culture were suspended in 5 ml of peptone water, incubated at 37 °C for 24 hours, and adjusted to match the turbidity of 0.5 McFarland standard. Following this, the suspension was inoculated onto a Muller-Hinton agar plate using a sterile cotton swab. The antibiotic discs (Himedia, Mumbai) were applied to the plate using sterile forceps, ensuring full contact. After 24 hours of incubation at 37 °C under aerobic and anaerobic conditions, the plates were examined. Finally, the isolates were classified as susceptible, intermediate, or resistant to each tested antibiotic, based on the CLSI guidelines (CLSI, 2016), by measuring the zone of inhibition around the antibiotic disc in millimeters. Intermediate results were considered resistant, as shown in Table 1 (Hombach *et al*., 2013; Maurer *et al*., 2014).

RESULTS

The results show that out of 64 bacterial isolates, 29 (45.3%) were Gram-negative, comprising *E. coli* (16, 43.2%), *Salmonella* spp. (4, 10.8%), *Proteus* spp (4, 10.8%), and *Klebsiella* spp. (5, 13.5%). Meanwhile, 35 (54.7%) isolates were Gram-positive, consisting of *Staphylococcus aureus* (10, 27.8%), *Streptococcus* spp (8, 22.2%), *Bacillus* spp (7, 19.4%), and *Staphylococcus* spp (10, 27.8%)(Table 1).

The antibiogram results of the bacterial isolates revealed varying patterns of resistance. Among the Gram-negative bacteria, *E. coli* (16 isolates) showed high resistance to Nitrofurantoin (68.8%), Ceftriaxone (75%), and Oxacillin (81.3%), but were highly susceptible to Amikacin (100%). *Salmonella* spp (5 isolates) and *Klebsiella* spp (4 isolates) also exhibited resistance to multiple antibiotics. In contrast, the Gram-positive bacteria, *Staphylococcus aureus* (10 isolates), displayed high resistance to Oxacillin (60%) and Erythromycin

(30%), but remained highly susceptible to Vancomycin (80%).

Table 1: Antimicrobial Agents, Concentration and their Breakpoint

Antimicrobial Agent	Concentration	Resistant (R)	Susceptible (S)
Ampicillin	10 µg	<21 mm	≥22 mm
Cephalothin	30 µg	<14 mm	≥15 mm
Cefotaxime	30 µg	<14 mm	≥15 mm
Ciprofloxacin	5 µg	<15 mm	≥16 mm
Clindamycin	30 µg	<14 mm	≥15 mm
Erythromycin	15 µg	<13 mm	≥14 mm
Gentamicin	10 µg	<12 mm	≥13 mm
Penicillin	10 U	<14 mm	≥15 mm
Sulfamethoxazole-Trimethoprim	25 µg	<10 mm	≥11 mm
Tetracycline	30 µg	<14 mm	≥15 mm
Vancomycin	30 µg	<14 mm	≥15 mm

AMP: ampicillin, CEF: cephalothin, CFT: cefotaxime, CIP: ciprofloxacin, CLI: clindamycin, DIC: dicloxacillin, ERI: erythromycin, GENE: gentamicin, PEN: penicillin, STM: sulfamethoxazole-trimethroprim, TET: tetracycline, VAN: vancomycin, CLO: chloramphenicol, CAR: carbenicillin, NET: netilmicin, NIT: nitrofurantoin, NOT: norfloxacin and AMI: amikacin (CLSI, 2016)

Table 2: Occurance of bacteria isolates swab samples from vagina of goats

Gram Characteristics	Bacteria Spp	Frequency (%)
Negative	*E. coli*	16 (43.2)
	Salmonella spp	4 (10.8)
	Proteus spp	4 (10.8)
	Klebsiella spp	5 (13.5)
Positive	*Staphylococcus aureus*	10 (27.8)
	Streptococcus spp	8 (22.2)
	Bacillus spp	7 (19.4)
	Staphylococcus spp	10 (27.8)

Table 3: Antibiogram for bacterial isolates from Vaginae of Goat Slaughter at Maiduguri Abattoir

Gram Characteristics	Bacteria	No. of Isolates	NIT	CEF	OXY	CIP	CLI	ERI	GEN	PEN	STM	AMI	VAN
Negative	*E. coli*	16	11	12	13	7	8	10	9	11	9	6	x
	Salmonella spp	5	4	3	5	4	4	4	3	4	5	4	x
	Klebsiella spp	4	3	3	2	3	2	3	3	3	3	2	x
Positive	*S. aureus*	10	8	7	6	8	7	7	7	8	8	x	8
	Streptococcus spp	0	0	0	0	0	0	0	0	0	0		
	Bacillus spp	0	0	0	0	0	0	0	0	0	0		

Keys: AMP: ampicillin, CEF: cephalothin, CFT: cefotaxime, CIP: ciprofloxacin, CLI: clindamycin, DIC: dicloxacillin, ERI: erythromycin, GENE: gentamicin, PEN: penicillin, STM: sulfamethoxazole-trimethroprim, TET: tetracycline, VAN: vancomycin, CLO: chloramphenicol, CAR: carbenicillin, NET: netilmicin, NIT: nitrofurantoin, NOT: norfloxacin and AMI: amikacin

DISCUSSION

This study isolated 64 bacterial isolates from vaginal swab samples of 25 non-pregnant Sahel goats, with 60% of the samples showing bacterial growth. The isolates comprised 35 Gram-positive bacteria (54.7%) and 29 Gram-negative bacteria (45.3%). Previous studies have reported varying bacterial isolation rates occurrence in different goat breeds and reproductive stages, ranging from 52% in Saanen goats of the reproductive stage (Manes *et al*., 2013) to 77% in Creole goats in conditions of gestational anestrus (Flores-Hernández *et al*., 2020). The bacterial isolation rate occurrences in this study fall within this range, highlighting the complexity of the vaginal microbial population. A total of 8 bacterial species were isolated, including *E. coli, Staphylococcus aureus, Staphylococcus spp., Salmonella spp., Streptococcus spp., Proteus spp., Bacillus spp*. and *Klebsiella spp*. This finding is consistent with previous studies, which also reported the isolation of multiple bacterial species (Al-Delemi, 2005; Bukar-Kolo *et al*., 2007; Manes *et al*., 2010; Al-Zubaidi *et al*., 2013; Mshelia *et al.*, 2014).

The vaginal microbiota (VM) of ruminants develops in response to environmental conditions and

physiological changes. However, any compromise to the integrity of the vaginal mucosa or alterations to the microbiota can trigger ascending infections of the urogenital tract, compromising the reproductive health of goats (Ababneh and Degefa, 2006). In this study, the predominant bacterial species in the vaginal microbiota of non-pregnant Sahel goats were Gram-positive bacteria, with *Staphylococcus aureus* being the most prevalent isolate, accounting for 27.8% of all Gram-positive isolates.

The complexity of the vaginal microbial population is further highlighted by the antibiotic resistance patterns observed in this study. The antibiogram results revealed an alarming pattern of antibiotic resistance. The Gram-negative bacteria, specifically *E. coli*, *Salmonella* spp, and *Klebsiella* spp, demonstrated high resistance to multiple antibiotics. Notably, *E. coli* showed high resistance to Nitrofurantoin (68.8%), Ceftriaxone (75%), and Oxacillin (81.3%), but remained highly susceptible to Amikacin (62.5%). *Salmonella* spp and *Klebsiella* spp also exhibited resistance to multiple antibiotics. In contrast, the antibiogram results of the Gram-positive bacteria, *Staphylococcus aureus*, revealed a different pattern of antibiotic resistance. *Staphylococcus aureus* displayed high resistance to Oxacillin (60%) and Erythromycin (30%), but remained highly susceptible to Vancomycin (80%), which is often considered a treatment of last resort for multi-drug-resistant *S. aureus* infections (Balami *et al.*, 2016).

These findings emphasize the importance of implementing effective antibiotic stewardship programs and infection control measures to mitigate the spread of antibiotic-resistant bacteria in the Sahel goat population.

CONCLUSION

This study isolated a total of 64 bacterial isolates from the vaginas microbiota of non-pregnant Sahel goats, revealing a complex microbial population. The key findings showed a high prevalence of *Staphylococcus aureus* (27.8% of all Gram-positive isolates) and a dominance of *E. coli* (43.2% of all Gram-negative isolates) and (58.6% of *E. coli* isolates were resistant to multiple antibiotics). Furthermore, concerning antibiotic resistance rates were observed among *E. coli* isolates, with 68.8% resistance to Nitrofurantoin, 75% resistance to Ceftriaxone, and 81.3% resistance to Oxacillin. These findings emphasize the importance of judicious antibiotic use and effective strategies to prevent and manage reproductive tract infections in Sahel goats

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