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Identification and Antimicrobial Susceptibility Profile of Preputial Bacterial Flora from apparently Healthy Bulls, Rams, and Bucks in Maiduguri, Northeastern Nigeria

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ABSTRACT

The aim of this study was to identify the preputial aerobic bacterial flora from bulls, rams and bucks and to determine their antimicrobial sensitivities. Preputial swabs were collected randomly from twenty each of clinically healthy post pubertal bulls, rams and bucks. Using standard bacteriological techniques, the following bacteria were identified; *Escherichia coli* (bulls 26.5%, rams 19.2% and buck 22.7%), *Staphylococcus aureus* (bulls 25.0 %, rams 26.0 % and bucks 24.2 %), *Streptococcus* (bulls 20.6%, rams 26.0 % and bucks 25.8%), *Salmonella* (bulls 14.7%), *Shigella* (rams 15.1% and bucks 15.2%) and *Klebsiella* (bulls 13.2%, rams 13.7 % and bucks 12.1%) species. There was no statistically significant difference ($p>0.05$) between the proportions of bacterial species and different animal species. In bulls, all bacterial isolates were susceptible to Ciprofloxacin (100%) and Levofloxacin (100%) while 60% of the bacterial isolates showed resistance to amoxicillin, ampiclox and streptomycin. In rams, all the bacterial isolates were susceptible to ciprofloxacin (100%) and gentamycin (100%) while 40% of bacterial isolates were resistant to chloramphenicol. In bucks, all bacterial isolates were susceptible to amoxicillin (100%), ampiclox (100%) and gentamycin (100%) while 20% bacterial isolates were resistant to streptomycin. This study shows that a few bacteria colonize the prepuce of ruminants in Maiduguri and there is need for preputial washing prior to breeding to reduce contamination of semen during natural mating or collection. Amoxiciillin, ampiclox, ciprofloxacin and gentamycin could be used as therapeutic agents to diseases that could be caused by the bacteria in prepuce of these animal species.

Keywords: Antimicrobial; Bacterial flora; Maiduguri; Prepuce; Susceptibility

INTRODUCTION

Food animals are extensively reared and they play an important role in trade and economies of developing countries by providing employment and income as well as a source of nourishment for millions of households worldwide (Herrero *et al*., 2013; Kimera *et al*., 2020). This has led farmers to an uncontrolled use of antimicrobials as a growth promotion feed additive to animals to enable them to increase their feed-to-muscle conversion rate. This has in no small measure contributed immensely to antimicrobial resistance (Ayukekbong *et al*., 2017; Adebowale *et al*., 2022). Despite the economic significance of livestock, many aspects of the reproductive biology and physiology remains to be elucidated including microbial community of certain body systems such as the preputial cavity (Wickware *et al*., 2020). A microbial community is an assemblage of microorganisms present in a defined environment. Microbial communities have an impact on the animal's health status and on possible disease outcomes as the animal's immune system is affected by changes in the micro-organisms and vice versa. So many studies have elucidated vaginal and uterine microbiota but that of male urogenital system remains to be studied.

The prepuce of animals is colonised by bacteria, and this makes collection of bacteria-free semen very difficult (Akhter *et al*., 2008; Rota *et al*., 2011; Yaniz *et al*., 2010; Meena *et al*., 2015). During breeding, mating activity in animals may lead to an increased contamination of the penis and prepuce and could lead to an increase in microbial load of semen especially during natural mating (Navya, 2012; Fatnassi *et al*., 2014). Akhter *et al*. (2008) and Navya (2012) had earlier opined that Semen in most animals is contaminated with aerobic preputial bacteria such as during collection or natural mating. Several bacteria including *Staphylococcus aureas*, *Brucella abortus*, *Proteus mirabilis Streptococcus pyogenes*, *Bacilus* sp, *Clostridium* sp, *E. coli*, *Corynebacteria* sp. have been frequently isolated from prepuce of animals such as Camel bulls, Rams and Stallions (Gouletsou *et al*., 2006; Zaid and Al-Zubaidy 2009; Wickware *et al*., 2020; Peter *et al*., 2023). High bacterial load in semen

reflects poor hygienic management of breeder males in different steps of animal husbandry, semen collection or handling (Patel *et al*., 2011). Preputial bacteria when left unchecked can get into semen and can compete with spermatozoa for nutrients and could cause impaired semen motility and semen morphology as well as causing other disturbs in semen quality parameters (Najee *et al*., 2012; Navya, 2012). Bacterial contamination could also compromise semen quality during storage, which would likely decrease the fertilization capability of spermatozoa (Harada and Asai, 2010). In infected female animals, the effect of preputial bacteria on reproductive processes could range from reduced conception rates, embryonic mortalities, abortion or still births (Meena *et al*., 2015).

Antimicrobial resistance is a major threat to global health as multidrug-resistant organisms are directly or indirectly responsible to an ever-increasing mortality in animals and humans. Common risk practices that have aided the occurrence of antimicrobial resistance in anticmicrobial agents is indiscriminate usage of antimicrobial agents (Adebowale *et al*., 2022) usually seen where livestock owners use such agents to treat microbial infections in animals without prior sensitivity tests or due a weak to non-existing antibiotic use policies in the country (Kabir, 2010; Kabir *et al*., 2004).

This investigation defines the reference microbial colonisation of the prepuce in bulls, rams and bucks in Maiduguri Northeastern Nigeria and their antimicrobial sensitivity pattern. The information that will be obtained from this study may assist in the determining factors that aid establishing of disease conditions on the superficial mucosal lining of the prepuce and penis.

MATERIALS AND METHODS

Study area and Animals

The study was done during the harmattan season between October and December 2021 in Maiduguri (Maiduguri Metropolitan Abattoir). Maiduguri is a city in Northeastern Nigeria. The city lies between 11°32' North and 11°40' North and longitude 13°20' East and 13°25' East between the Sudan Savannah and Sahel Savannah vegetation zones (Udoh, 1981). Clinically healthy male adult bulls (n=20, approx. 300kg, aged 3 years and above), Balami rams (n=20, approx. 33kg, aged 2 years and above) and Sahel bucks (n=20, approx. 25kg, aged 2 years and above) used as trade animals brought for slaughter from the surrounding states of Yobe and Adamawa as well as neighbouring Countries of Niger and T'Chad were used for this study.

Sample collection and identification of bacteria

Bulls (n=20), rams (n=20), and bucks (n=20) were physically restrained using ropes and placed on lateral recumbency ready for slaughter. The preputial hair was clipped where necessary and disinfected using mild disinfectant (Savlon®). The preputial opening was gently opened and a sterile swabs stick (Evepon Sterile Swab Sticks®, Evepon Industries Limited, Anambra, Nigeria) was inserted and rotated. The swab stick was then withdrawn and placed in nutrient broth then placed in a flask. This was then transported to the Bacteriology laboratory at the Department of Veterinary Microbiology and Bacteriology, Faculty of Veterinary Medicine, University of Maiduguri within 30 minutes after collection.

Isolation and identification

The preputial swab samples were brought to the laboratory already inoculated in nutrient broth. A loop was taken from this media and streaked on Blood agar, Eosin Methlyne Blue, Mannitol Salt Agar, Salmonella Shigella Agar, McConkey Agar. These were incubated at 37°C for 24 h aerobically. In cases where multiple colonial growths were seen, such colonies were further sub-cultured to obtain pure cultures. Biochemical tests were carried out to further confirm the bacteria as described according to standard procedures (Cowan and Steel, 1993; Holt *et a*l., 1994).

Antimicrobial susceptibility test

The antibiotic susceptibility test for the identified bacteria were applied with multi discs containing Amoxycillin (30 µg), Ampiclox (30 µg), Levofloxacin (10 µg), Chloramphenicol (20 µg), Ciprofloxacin (10 µg), Gentamycin (10 µg) and Streptomycin (30 µg). The antimicrobial susceptibility test was performed according to the Kirby Bauer disc diffusion method (Bauer *et al*., 1966).

Statistical analysis

All data generated from this study was compiled and managed using Microsoft Excel spread sheet (Windows 11). The data were summarised and presented using frequency distribution tables. Chi square analysis was used to determine statistical significance in the frequency of occurrence of bacteria in all species mentioned using Statistical Package for Social Scientist SPSS IBM v 27. Values at $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

Bacteria isolated from prepuce of bulls, rams and buck in Maiduguri, Northeastern, Nigeria

Cultures of preputial swabs from the bulls, rams and bucks yielded 207 bacterial isolates. The number of isolates/male ranges from two to five per animal. Six distinct bacterial species were identified from which two were gram positive (*S. aureus* and *Streptococcus spp*) while four were gram negative (*Escherichia coli, Shigella, Klebsiella* and *Salmonella* species). Furthermore, no gross preputial abnormality was observed in any of the animals from which preputial swab samples were collected in this study. *Escherichia coli* (26.5%) and *Staphylococcus aureus* (25.0%) were the most frequently isolated bacteria from prepuce of bulls. Other bacterial isolates were *Streptococcus* species (20.6%), *Salmonella* species (14.7%) and Klebsiella species (13.2%). The most frequently isolated bacteria from the prepuce of rams were *Staphylococcus aureus* (26.0%), *Streptococcus* species (26.0%), *Escherichia coli* (19.2%), *Shigella* species (15.1%) and *Kliebsiella* species (13.7%). In bucks, *Streptococcus* species was the frequently isolated bacteria (25.8%), followed by *Staphylococcus aureus* (24.2%), *E. coli* (22.7%), *Shigella* (15.2%) and *Kliebsiella* species (12.1%) (Table 1).

Antibiotic sensitivity profile of bacterial isolates from prepuce of bulls, rams and bucks in Maiduguri, Northeastern Nigeria.

The antibiotic susceptibility test of these isolated bacteria is presented in Table 2. The table shows that *E. coli* isolated from prepuce of bulls showed resistance to Gentamycin and streptomycin. Most bacteria isolated from prepuce of Rams and Bucks show susceptibility to the antimicrobial agents used in this study.

Table 1: Bacteria isolated from prepuce of Cattle Sheep and Goats in Maiduguri, North-eastern Nigeria**.**

Bacteria	**Number of Isolates**				
	Bull	**Ram**	**Buck**	X^2	***p* value**
	n (%)	**n (%)**	**n (%)**		
Escherichia coli	18 (26.5)	14 (19.2)	15 (22.7)	2.553	0.28
Staphylococcus aureus	17 (25.0)	19 (26.0)	16 (24.2)	2.019	0.36
Streptococcus spp	14 (20.6)	19 (26.0)	17 (25.8)	4.560	0.10
Salmonella spp	10 (14.7)	-	-		
Shigella spp	-	11 (15.1)	10 (15.2)	0.100	0.75
Klebsiella spp	9 (13.2)	10 (13.7)	8 (12.1)	0.404	0.82
Total	**68 (100)**	**73 (100)**	**66 (100)**		

Table 2: Antibiotic Susceptibility Profile of Bacterial isolates from prepuce of Bulls, Rams and Bucks in Maiduguri, North-eastern Nigeria.

	Antibacterial agent																				
Type of animal/	Amoxicillin			Ampiclox			Chloramphenicol			Ciprofloxacin			Gentamycin			Levofloxacin			Streptomycin		
Bacterial isolate	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
Bulls																					
E. coli	-	18	-	-	-	18	-	-	18	-	-	18	18	-	-	-	-	18	18	-	-
S. aureus	-	-	17	17	-	-	-	-	17	-	-	17	-	-	17	-	-	17	-	-	17
Strept. spp	14	-	-	14	-	-	-	-	14	-	-	14	-	-	14	-	-	14	14	-	-
Salmonella	10	-	-	10	-	-	10	-	-	-	-	10	9	-	10	-	-	10	10	-	-
Kleibsiella	9	-	-	-	9	-	9	-	-	-	-	9		-	-	-	-	9	-	9	-
Rams																					
Streptococcus	-	19	-	-	-	19	-	-	19	-	-	19	-	-	19				-	19	-
Staph aureus	-	19	-	-	-	19	-	-	19	-	-	19	-	-	19				-	-	19
Escherichia coli	-	-	14	-	-	14	-	14	-	-	-	14	-	-	14				-	14	-
Shigella	-	-	11	-	-	11	11	-	-	-	-	11	-	-	11				11	-	-
Klebsiella	-	-	10	10	-	-	10	-	-	-	-	10	-	-	10				-	8	-
Bucks																					
Streptococcus	-	-	17	-	-	17	-	-	17	-	-	17	-	-	17				-	-	17
Staph aureus	-	-	16	-	-	16	-	-	16	-	16	-	-	-	16				-	16	-
Escherichia coli	-	-	15	-	-	15	-	15	-	-	-	15	-	-	15				15	-	-
Shigella	-	-	10	-	-	10	-	-	10	-	10	-	-	-	10				-	-	10
Klebsiella	-	-	8	-	-	8	-	8	-	-	-	8	-	-	8				-	-	8

S= Sensitive, I= Intermediate, R= Resistant

This study was designed to investigate the presence of bacteria in the preputial cavity of bulls, rams and bucks in Maiduguri, Northeastern Nigeria. The animals sampled were semi-intensively managed. *Escherichia coli* (26.5%), *Staphylococcus aureus* (25.0%), *Streptococcus* spp (20.6%), *Salmonella* spp (14.7%) and *Klebsiella* (13.2%) were isolated from prepuce of bulls. Similar bacterial isolates were isolated from rams and bucks; *S. aureus (*rams 26.0 % and bucks 24.2 %*), E. coli* (rams 19.2% and buck 22.7%), *Streptococcus* (rams 26.0 % and bucks 25.8%), *Shigella* (rams15.1% and bucks 15.2%) and *Klebsiella* (rams 13.7 % and bucks 12.1%) species. Very few studies have elucidated on the microbial community of the prepuce of animals in this region. The results of this study were found to be similar with other studies that investigated microbial agents in the reproductive tract of animals. A recent study has documented the presence of *E. coli*, *Streptococcus* species, *Staphylococcus aureus, Klebsiella* and *Shigella* species among camel bulls in Maiduguri (Peter *et al*., 2023). They are also like vaginal flora from other healthy animals in the same study area (Bukar-Kolo *et a*l., 2007; 2016; Mshelia et al., 2014). Several other studies have long reported similar bacteria in preputial cavities of animals (Fache *et al*., 1985; Eaglesome *et al*., 1992).

It is noteworthy to put into consideration and to examine the potential source of these isolated bacteria. The major sources of bacteria that colonize the prepuce have been earlier identified to be from the soil mixed with faeces when the animal is on sternal recumbency or vagina of cows during coitus (Wickware *et al*., 2020). Other factors that could be related to source of preputial bacteria are the length of preputial hair or the terminal urethra (Romano *et al*., 2022). However, the conditions within the preputial cavity might have an even greater influence on the species of bacteria that inhabit the preputial cavity than where they are in the soil, faeces or urine. Just as the prepuce is, the cow vagina and upper respiratory tract are other similar mucosal environments where such bacteria have been isolated in other animals' species. Although these mucosal environments are not essentially connected, the environmental conditions could be the main reason why these bacteria colonize these locations. In a detailed investigation, Wikware *et al*. (2020) also opined that bacteria-bacteria interactions play a significant role in the occurrence of preputial bacteria.

The bacteria that were isolated in this study were from apparently healthy animals without gross preputial pathologies suggesting these bacteria are commensals. However, these bacteria could easily contaminate ejaculated semen during natural mating or semen collection. They also have the potential to cause pathological changes when they contaminate semen and can even establish venereal diseases in infected animals (Givens, 2018). Bacterial contaminants in semen affect seminal characteristics as well as fertility to a large extent. Contaminated semen could cause a chain of pathological changes that may include changes in semen pH, a reduction in sperm motility, a high incidence of sperm clumping and an increased proportion of altered spermatozoa acrosome (Peter *et al*., 2023).

Bacterial isolates obtained from this study have the potential to cause and maintain venereal diseases in animal populations. Such diseases are mainly spread through coitus and infraction is usually transmitted from an infected female to a susceptible male or from an infected male to a susceptible female. An unidentified male can acquire infection after mating with an infected female and thus begin to spread such infection to susceptible females during normal mating within a short period of time. In an unusual case, the use of infected semen could lead to spread of venereal disease during artificial insemination among animals. An infected immunocompetent animal can clear such an infection by mounting adequate cellular immune response. However, in some animals, the defence to such microbial infection may partially or completely fail thus allowing the establishment of infection and could lead to infertility or sterility. Flushing the preputial cavity with an isotonic solution or a mild antiseptic just before semen collection or regular washing of the preputial cavity with mild antiseptic prior to natural mating is an effective way to reduce preputial bacterial load in animals.

Antimicrobial agents have been used *in vivo* as therapeutic agents against bacterial agents. Nevertheless, bacteria have developed resistance to antimicrobial agents (Chander and Raza, 2013). *In vitro* antimicrobial sensitivity in this study revealed most bacterial isolates from the sampled animals shows sensitivity to common antimicrobials. In bulls sampled, all bacterial isolates were susceptible to Ciprofloxacin (100%) and Levofloxacin (100%) while 60% of the bacterial isolates showed resistance to amoxicillin, ampiclox and streptomycin. In rams, all the bacterial isolates showed susceptibility to ciprofloxacin (100%) and gentamycin (100%) while 40% of bacterial isolates were resistant to chloramphenicol. In bucks, all bacterial isolates were susceptible to amoxicillin (100%), ampiclox (100%) and gentamycin (100%) while 20% bacterial isolates were resistant to streptomycin. This agrees with earlier studies where Gentamycin and Ciprofloxacin were found to be therapeutic agent for venereal diseases in Rams and Bucks (Ajala *et al*., 2011; Mshelia *et al*., 2014). *E. coli, Streptococcus, Salmonella* and *Klebsiella,* and *Shigella* isolates showed resistance to a few antimicrobial agents used in this study. Resistance of bacteria to antimicrobial agents is currently a global public health issue. A global projection predicts the increase of deaths linked to antimicrobial resistance may likely rise to 10 million deaths by 2050 (Ma *et al*., 2020; Adebowale *et al*., 2022). Antimicrobial resistance is thought to arise through bacterial acquisition of antimicrobial resistant determinants from environmental reservoirs, modifying enzymes or through disseminated use of antimicrobials (Poole, 2005; Economou and Ghousia, 2015; Igbinosa *et al*., 2022).

Conclusion

The study shows that *E. coli, S aureus, Streptococcus* spp, *Salmonella* spp, *Klebsiella and shigella* are frequently isolated from the prepuce of bulls, rams and bucks in Maiduguri Northeastern Nigeria. These bacteria were isolated from apparently healthy animals and are therefore regarded as commensals. However, they could also serve as a potential source of infection and could lead to infertility in animals. Preputial samples should continue to be collected from animals and bacterial isolated therein be characterized to gain a complete picture of the microbial community of the prepuce and their antigenic makeup. It is strongly advised that the prepuce of ruminants in this location be washed prior to semen collection and before natural mating to reduce bacterial load in semen of these species of animals. It is also essential to develop and enforce a national antimicrobial use policy through the one health intervention to improve the judicious use of antimicrobials and antimicrobial resistance containment.

Author Contribution

IDP and JS conceived the idea and designed the experiment. DI, RIA and SOA collected samples and together with MMM and MB run the laboratory tests. IDP wrote the first draft and was revised by JS, RIA, SOA. RIA AND SOA performed the statistical analysis. All authors read and endorsed the revised manuscript

Conflict of Interest

The authors have no conflict of interest to declare.

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