

Microbiome from prepuces of household cats and hunting dogs in Maiduguri, Northeastern Nigeria, and antimicrobial susceptibility pattern of aerobic bacteria isolated from the prepuces

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Abstract

This study identified bacteria isolated from the preputial cavity of household cats and hunting dogs in Maiduguri, Northeastern Nigeria, and also determined their susceptibility pattern to common anti-microbial agents. Preputial swab samples were collected from forty household cats and thirty-two hunting dogs. Following culture and biochemical analyses, eight bacterial species were identified, and these included *Bacillus* spp. (cats – 77.5%, dogs – 56.3%), *Klebsiella* spp. (cats – 37.5%, dogs – 96.9%), *Shigella* spp. (cats – 30.0%), *Staphylococcus* spp. (cats – 20.0%, dogs – 59.4%), *Escherichia coli* (dogs – 25.0%), *Salmonella* spp. (cats – 15.0%, dogs – 12.5%), *Enterobacter* spp. (cats – 12.5%), and *Proteus* spp. (cats – 5.0%). There was a high frequency of isolation of *Bacillus* spp. in both cats and dogs, but *Klebsiella* spp. and *Staphylococcus* spp. were isolated in high frequency only in the dogs. The frequency of isolation of *Klebsiella* spp. and *Staphylococcus* spp. was significantly ($p < 0.05$) higher in dogs than in cats. The resistance at isolate level was low for all the antimicrobials tested; however, some bacterial isolates were found to be resistant to augmentin, amoxicillin and gentamicin. This study showed that multiple bacterial species colonize the preputial cavities of household cats and hunting dogs in Maiduguri, Nigeria. These bacterial isolates have the potential to cause bacterial diseases in the prepuce of such affected animals and can possibly lead to reproductive disorders that may adversely affect the fertility of affected animals. Regular washing of the prepuce in these species of animals prior to breeding is recommended.

Keywords: Microbiome; Prepuce; Household cats; Hunting dogs; Maiduguri, Nigeria; Antimicrobial susceptibility.

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Introduction

Dogs are believed to be the earliest domesticated animal and were originally utilized mostly for hunting in ancient times (Zakariah *et al.*, 2017; Perri *et al.* 2021; Holland *et al.*, 2022; Mustapha *et al.*, 2023). Cats and dogs have become the most sought companion animals due to their intellect, cognitive behaviour and ability to communicate non-verbally with people (Sanjana *et al.*, 2022)

Contact between humans and pet animals has been increasing in recent years, and this may possibly result in the spread of infectious agents (Ghasemzadeh and Namazi, 2015; Overgaauw *et al.*, 2020; Rey *et al.*, 2020; Kinunen *et al.*, 2022). Cats and dogs are susceptible to various bacterial diseases that may affect their fertility (Chaudhary *et al.*, 2024). The emergence of antimicrobial resistance amongst bacteria has recently been a major concern in veterinary and human medicine, with significant implications for the health of animals and humans (Awosile *et al.*, 2018).

Microorganisms that inhabit specific parts of the body (e.g. preputial cavities) are collectively called microbiome (Holst *et al.*, 2003; Rey *et al.*, 2020). The role of such bacteria in many reproductive disturbances in cats and dogs has been earlier reported (Pretzer, 2008; Graham and Taylor, 2012; Stefanetti *et al.*, 2018). It is important to note that bacteria exhibit complex virulence mechanisms possibly responsible for different nosocomial and community infections in humans and different species of animals (Rey *et al.*, 2020). As a consequence, owners of animals with reproductive disorders often want to have their animals treated with antimicrobials. Owners of male animals sometimes require that the selected females be treated with antimicrobials especially when bacteria are detected in vaginal swabs to reduce the risk of transmission of bacteria to

the males despite the fact that microbial populations are present on the preputial mucosa (Johnson *et al.*, 1996). While majority of bacterial pathogens exist as commensals and protect the host immune system from other pathogens, a few may cause diseases (Sanjana *et al.*, 2022).

The prepuce is the anatomical skin covering the glans penis, protecting the penis by reducing drying, irritation and contamination. In small animals, the prepuce forms a complete sheath around the cranial end of the penis covering the retracted, inactive end of the penis (Levine and Jacobs, 2022). The prepuce has been reported to serve as a reservoir of microorganisms (Agartan *et al.*, 2005). Prominent sources of these preputial microorganisms are the soil and the female genital tract (Waheed *et al.*, 2022). Some of these microorganisms can be pathogenic and therefore might predispose affected animals to reproductive disorders and possibly infertility, thus allowing the transmission of venereal infections from infected males to females and vice versa.

Cats and dogs are exposed to different pathogens from diverse sources, such as the environment, fomites, humans and other animals. Antimicrobial agents are commonly used as treatment for such pathogens. However, increased occurrence of treatment failures with its concomitant increased patient management cost has been associated with antimicrobial resistance in companion animals (Cummings *et al.*, 2015; Awosile *et al.*, 2018). There have been no reports in available literature on the microbiome inhabiting the prepuce of dogs and cats in Maiduguri, Nigeria, and the antimicrobial susceptibility of such microbiome. The present study identified the aerobic bacteria of the prepuce in clinically healthy adult household cats and hunting dogs in Maiduguri Northeastern Nigeria, and also determined the susceptibility/sensitivities of isolated bacteria to common antibiotics.

Materials and Methods

Study Area and Animals: The study was done between October and December 2023 in Maiduguri, Nigeria, using the facilities of Microbiology Laboratory, Faculty of Veterinary Medicine, University of Maiduguri. Maiduguri lies between latitude 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 post-pubertal male cats (n = 40, aged 4 months and above) brought to the University of Maiduguri Veterinary Teaching Hospital for routine veterinary care and Nigerian indigenous breed of hunting dogs (n = 32, aged 6 months and above) were randomly sampled for this study.

Ethical approval for this study was obtained from the Animal Care and Use Committee of the Faculty of Veterinary Medicine, University of Maiduguri, Nigeria (FVM699/15/10/1/116). Informed consent for obtaining preputial swab samples from the cats and dogs were obtained from the animal owners before samples were collected from them, and the animals were handled in accordance with the internationally acceptable principles for animal use and care (NASEM, 2020).

Sample Collection: The cats were restrained on an examination table on lateral recumbency with the dorsum of the cat against the handler. The animal's fore and hind limbs were both restrained by the handler. For dogs, they were restrained by applying a muzzle on the mouth and physically restrained in lateral recumbency. Preputial hairs were clipped where necessary in both species of animals to expose the preputial opening and this was disinfected using mild disinfectant (Savlon®, Johnson and Johnson Pty Limited, South Africa). Applying little pressure and gentle twisting opened the preputial orifice and a sterile swab 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, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria within 30 minutes of collection.

Isolation and Identification of Bacteria: 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 Methylene Blue, Mannitol Salt Agar, *Salmonella Shigella* Agar, McConkey Agar. These were incubated aerobically at 37°C for 24 hours. 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 following standard procedures (Cowan and Steel, 1993; Holt *et al.*, 1994).

Antimicrobial Susceptibility Tests: The antimicrobial susceptibility test for the identified bacteria was done with multi discs containing Amoxicillin (30 µg), Augmentin (30µg), Ciprofloxacin (10 µg), Erythromycin (10µg), Gentamycin (10 µg), Pefloxacin (10µg), Septrin (30µg), Spafloxacin (10µg), Streptomycin (30 µg) and Tarivid (10µg). The antimicrobial susceptibility test was performed, following the Kirby Bauer disc diffusion method (Bauer *et al.*, 1966).

Statistical analysis: All data generated from this study were compiled and managed using Microsoft Excel spread sheet (Windows 11). The data were summarized and presented using frequency distribution tables. Chi square test was used to comparatively analyze the frequency of occurrence of bacteria between cats and dogs. Values at $p < 0.05$ were considered significant.

Results

Age of the animals used for the study: A high percentage (57.5%) of the cats used for the study were 7 – 9 months of age, with 32.5% of them being 10 – 12 months of age (Table 1). Cats between 4 – 6 months of age constituted 7.5% of the sample population while those more than 12 months of age were 2.5% of the sample population (Table 1). For the dogs, three hunting dogs (9.3%) were found to be less than one year of age; four dogs (12.5%) were between one and two years of age; nine (28.1%) were found to be between three and four years of age; ten (31.2%) were between five to six years of age; and six (18.8%) were above 7 years of age (Table 1).

Bacteria isolated from the cats and dogs: A total of seven bacterial species were isolated from preputial cavities of the 40 cats used for the study (Table 2). Some cats were found to harbor multiple bacterial species. The bacteria isolated from the cats and their frequency of isolation were: *Bacillus* species – 31 cats, *Klebsiella* species – 15 cats, *Shigella* species – 12 cats, *Staphylococcus* species – 8 cats, *Salmonella* species – 6 cats, *Enterobacter*

species – 5 cats, and *Proteus* species – 2 cats (Table 2).

For the 32 hunting dogs, five bacterial species were isolated from their preputial cavity, and most of the dogs harbored multiple bacteria species (Table 2). The bacteria isolated from the hunting dogs and the frequency of isolation were: *Klebsiella* spp – 31 dogs, *Staphylococcus* spp – 19 dogs, *Bacillus* spp – 18 dogs, *Escherichia coli* – 8 dogs, and *Salmonella* spp – 4 dogs (Table 2).

Antibiotic sensitivity of the bacterial isolates:

The bacterial isolates obtained from cats were found to be mostly resistant to Augmentin and Amoxicillin (Table 3). Similarly, bacterial isolates obtained from hunting dogs in this study were mostly resistant to augmentin and gentamicin (Table 4). Generally, the prevalence resistance at isolate level was low for all the antimicrobials tested. Resistance was found to be most prevalent for augmentin, amoxicillin, spafloxacin and streptomycin in samples obtained from cats (Table 3). In samples collected from dogs, resistances were recorded for amoxicillin, augmentin and gentamicin (Table 4).

Table 1. Age distribution of cats and hunting dogs from which samples were collected to evaluate preputial microbiome in Maiduguri, Northeastern Nigeria.

Animal	Age	Number of animals, with % in brackets
Cats	4 – 6 months	3 (7.5%)
	7 – 9 months	23 (57.5%)
	10 – 12 months	13 (32.5%)
	≥ 12 months	1 (2.5%)
	Total	40 (100%)
Hunting dogs	≤ 1 year	3 (9.3%)
	1 – 2 years	4 (12.5%)
	3 – 4 years	9 (28.1%)
	5 – 6 years	10 (31.2%)
	≥ 7 years	6 (18.8%)
	Total	32 (100%)

Table 2. Distribution of bacteria isolated from the preputial cavity of household cats (n = 40) and hunting dogs (n = 32) in Maiduguri, Northeastern Nigeria.

Bacterial isolates	Number of cats, with % in brackets.	Number of dogs, with % in brackets.	P-value
<i>Bacillus species</i>	31 (77.5%)	18 (56.3%)	0.055
<i>Klebsiella species</i>	15 (37.5%)	31 (96.9%)	0.000
<i>Shigella species</i>	12 (30.0%)	-	-
<i>Staphylococcus species</i>	8 (20.0%)	19 (59.4%)	0.001
<i>Escherichia coli</i>	-	8 (25.0%)	-
<i>Salmonella species</i>	6 (15.0%)	4 (12.5%)	0.761
<i>Enterobacter species</i>	5 (12.5%)	-	-
<i>Proteus species</i>	2 (5.0%)	-	-

Table 3. Antimicrobial susceptibility pattern of preputial bacteria isolated from preputial cavity of cats (n = 40) in Maiduguri, Northeastern Nigeria.

Antibiotics	<i>Bacillus</i>	<i>Enterobacter</i>	<i>Salmonella</i>	<i>Shigella</i>	<i>Klebsiella</i>	<i>Proteus</i>	<i>Staph.</i>
Amoxicillin	–	R	R	I	R	R	–
Augmentin	–	R	R	R	R	R	–
Ciprofloxacin	–	I	S	–	S	I	–
Erythromycin	I	–	–	–	–	–	I
Gentamycin	S	–	–	–	–	–	S
Pefloxacin	–	R	S	S	S	S	S
Septtrin	S	S	R	S	S	S	S
Sparfloxacin	–	R	R	–	S	I	–
Streptomycin	S	R	R	–	–	I	S
Tarivid	–	R	S	–	S	S	–

[R = Resistant, I = Intermediate, S = Sensitive, – = No visible reaction].

Table 4. Antimicrobial susceptibility pattern of bacteria isolated from prepuce of hunting dogs (n = 32) in Maiduguri, Northeastern Nigeria.

Antibiotics	<i>Bacillus</i>	<i>Salmonella</i>	<i>E. coli</i>	<i>Klebsiella</i>	<i>Staphylococcus</i>
Amoxicillin	S	R	–	–	R
Augmentin	–	R	R	R	–
Ciprofloxacin	S	S	–	S	R
Erythromycin	I	–	–	–	R
Gentamycin	–	R	R	R	–
Pefloxacin	S	–	–	–	S
Seprtin	S	–	R	S	R
Sparfloxacin	–	R	S	–	–
Streptomycin	S	R	S	R	S
Tarivid	–	–	S	–	–

[R = Resistant, I = Intermediate, S = Sensitive, – = No visible reaction]

Discussion

The finding in the present study that most (57.5%) of the cats sampled were between 7 to 9 months of age while the dogs sampled were mostly between 5 – 6 years of age old (31.2%) implies that most of the animals sampled for this study have attained puberty and are sexually active.

In the present study, gross preputial abnormalities were not observed in any of the animals sampled. The bacteria species that were isolated from the prepuce of cats and dogs in this study could thus be regarded as commensals as there were no pathological lesions seen in any of the animals. There is a dearth of information regarding the bacterial composition in the prepuce of cats and dogs in this region to enable comparison with the results of this study. However, a few studies have shown similar bacteria colonizing the prepuce of other species such as camel bulls, cattle bulls, bucks, rams and stallions within

this region (Peter *et al.*, 2023; 2024 and 2025). The results of this study is also comparable with the works of Holst *et al.* (2003) who also isolated *E. coli* and *Staphylococcus* species from the prepuce of cats.

It is thought that the major source of bacteria that colonize the prepuce in these animals could be from the soil and that these animals get these bacteria when they are on sternal recumbency or from the vagina of females during mating. 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. Just as the prepuce is, the vagina and upper respiratory tract are other similar mucosal environments where similar bacteria have been isolated in other species. Although these mucosal environments are not essentially

connected, the environmental conditions could be the main reason why these bacteria colonize these locations.

These bacteria isolated from the prepuce 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 lead to 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).

There is a growing concern about zoonotic transmission of antimicrobial resistance and this has led to questions surrounding the appropriateness of the use of some antimicrobial agents in animals (Ma *et al.*, 2021). Though most of these concerns are focused on food animals, nonetheless, information on antimicrobial resistance in companion animal can be utilized for directing rational early therapeutic decisions, developing antimicrobial stewardship guidelines and public policy.

The results of this study also suggest that household cats and hunting dogs may be considered as potential reservoirs of some bacteria that are found to be resistant to common antibacterial agents which may be a concern due to the close co-existence of these animals and their owners (Rey *et al.*, 2020). There may be reason to worry when dealing with hunting or asymptomatic cats and dogs, since their behavioral peculiarities (licking the genitalia, licking the body, sniffing the environment for territorial demarcation) may accelerate the transmission of microorganisms and even spread genes of resistance to their owners, other animals and even to the

environment (Rey *et al.*, 2020). It is therefore necessary for health education and enlightenment programmes to be conducted so as to raise awareness of the populace about the different situations that may favour the dissemination of microorganisms and their resistance genes.

Conclusion: Results of this study show that *Bacillus*, *Kliebsiella*, *Shigella* and *Staphylococcus* species were frequently isolated bacteria from the prepuce of household cats and hunting dogs in Maiduguri, Northeastern Nigeria. These isolates could be regarded as commensals as there was no preputial pathology recorded in any of the animals. The antimicrobial resistance at isolate level was low for all the antimicrobials tested, however, some of the bacterial isolates obtained from cats and dogs in this study were mainly found to be resistant to augmentin, amoxicillin and gentamicin. A more detailed study with a larger sample size and the use of molecular techniques is recommended to fully elucidate the diversity of bacteria that inhabit the prepuce, the pathologies associated with their presence and their antimicrobial susceptibility pattern.

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Conflict of Interest

The authors have no conflict of interest to declare.

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