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Pathogenic Bacteria Isolated from Cranial Orifices of Apparently Healthy Dogs (*Canis lupus familiaris*) in Makurdi, Benue State, Nigeria

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Abstract

Dogs, in Makurdi, Benue State, Nigeria, kept as pets, companions, and for security, live and interact very closely with humans, and since they harbor microorganisms, including bacteria, humans are often at risk of contracting pathogens from them. To determine this risk to dog owners in Makurdi, 100 samples were taken by swabs from the nostrils, eyes, mouths, and ear canals of 25 privately owned, apparently healthy dogs, and cultured in Tryptic Soy Broth. Isolates included *E. coli*, *E. faecalis*, *Klebsiella* sp., *Micrococcus* sp., *Neisseria* sp., *P. multocida*, *Salmonella* sp., *Staphylococcus* sp., and *Streptococcus* sp. The most prevalent bacteria were *Staphylococcus* spp., 88 (35.5%), while the least were *Micrococcus* and *Neisseria* spp., 2 (0.8%). Though the younger (0 – 12 months) and lighter dogs (0 - 12 kgs) had higher isolates, 114 (45.97%) and 84 (33.9%), respectively, age, breed, sex, and weight did not significantly affect bacterial isolates. By breed, Lhasa dogs harbored more bacteria, 60 (24.2%), compared with 12 (4.8%) from Eskimos. More bacteria were isolated from the oral cavity - 71 (28.6%), while the least were from the nasal cavities - 58 (23.4%). This difference was, however, not statistically significant. The bacteria isolated were all either pathogenic or opportunistic pathogens of man. Given the right conditions, they could cause serious or even life-threatening human infections. It is therefore important that dog owners give priority to their health and hygiene to help safeguard their own well-being.

Keywords: Bacteria, Cranial Orifice, Dogs, Healthy, Makurdi

Introduction

Dogs (*Canis lupus familiaris*) are domesticated descendants of the wolf and have been kept as pets for over 1,400 years by humans [1]. The dog, more than any other animal, has a close relationship with, and in most cases is also treated with, antimicrobials used by humans [2].

Dogs are kept by all categories of people irrespective of socioeconomic background and geographical location [3]. Although data is scarce in Africa, the increase in human population is accompanied by an increase in dog population [4].

Dogs harbor bacteria that, though not pathogenic to them, may be reservoirs of human infection [5]. Dogs transmit viral and bacterial diseases to humans through saliva, contaminated feces, urine, or by direct contact. The most important bacteria transmitted are *Salmonella* spp., *Yersinia* spp., *Capnocytophaga* spp., *Coxiella burnetii, Staphylococcus intermedius,* and *Leptospira* spp., in addition to Methicillin-Resistant *Staphylococcus aureus* (MRSA) and *E. coli* [6, 7].

In a recent 2019 case, a woman with an open cut was reportedly licked by her dog. She subsequently developed life-threatening sepsis and had to undergo a quadruple amputation. The sepsis was attributed to a rare bacterium, *Capnocytophaga canimorsus*, commonly found in the saliva of dogs and cats. Similarly, a man, in 2018, also underwent amputations after contracting *C. canimorsus* from his dog [8, 9, 10].

This study sheds light on canine health in Makurdi, improves veterinary care, contributes to comparative microbiome research, advances local veterinary science, and the broader field of canine microbiota research and its implications for human health.

Materials and Methods

Area of study

Makurdi (Figure 1), the capital of Benue State, lies between latitude 7° 43' 50" N and longitude 8° 32' 10" E. Makurdi shares boundaries with Guma Local Government to the Northeast, Gwer to the South, Gwer-West to the west, and Doma Local Government Area of Nasarawa State to the North-West. The indigenous tribes are the Tiv, Idoma, Igede, and Jukun; however, being cosmopolitan, it is home to different tribes like Agatu, Etulo, Igbo, Hausa, and Igala, etc., with an estimated population of 500,797 and a landmass of 16 km radius [11].

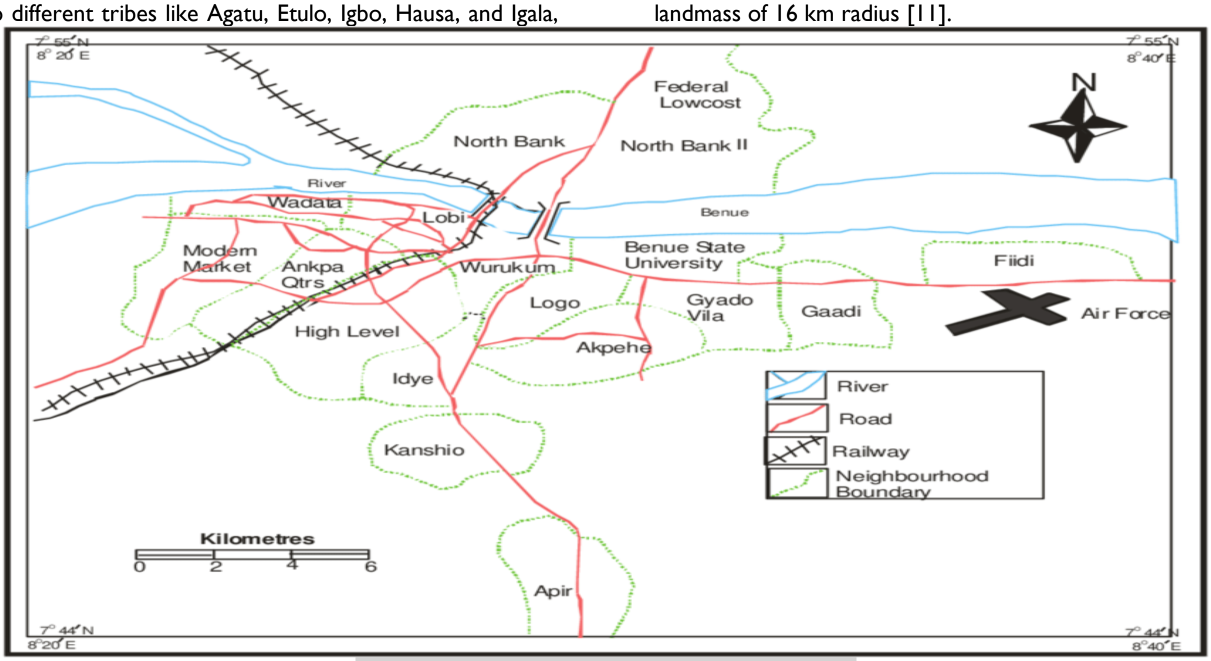

Figure 1: Map of Makurdi showing study area

Animals and specimen collection

A total of 100 samples were collected from 25 apparently healthy dogs in Makurdi between July and September 2024. All dogs were privately owned but had outdoor access. Physical examination was performed on each dog. The ages, weights, sexes, and dog breeds were noted. Specimens were collected from the mouths, noses, ears, and eyes by passage of sterile swabs against the animals' teeth, gums, tongue, and hard palate for oral samples. Nostril samples were collected by carefully inserting and rotating sterile swabs into the nostrils. Eye samples were collected after moistening the swab in normal saline. The lower eyelid was gently everted to expose the membrane, which was gently swabbed, avoiding the cornea. No anaesthetics or other drugs were administered prior to sample collection. Ear samples were collected by firmly rotating a swab stick towards the inner ear canal. All swabs were properly sealed, labeled, placed in ice-packed containers, and transported to the microbiology laboratory of Benue State University for analysis.

Processing of samples

Approximately 9 mLs of Tryptic Soy Broth (TSB) was dispensed into universal bottles with appropriate sample labels. The swabs were inoculated into the bottled broth and incubated aerobically for 8 hours at 37°C. Thereafter, the TSB cultures were inoculated onto 5% blood agar and incubated aerobically at 37°C for 24 hours. Following incubation, the plates were examined macroscopically for bacterial colonies by identifying colonial morphology (color, consistency, size, and smell), and microscopically, after Gram staining.

Biochemical tests

Biochemical tests used to identify bacteria were carbohydrate utilization, carbohydrate metabolism, enzyme production, Triple sugar iron agar (TSIA), catalase, indole, oxidase, and coagulase tests [12].

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 20 was used for analysis. Chi-square (χ^2) determined the significant relationships between variables at a 0.05 significance level.

Results

Frequency and distribution of bacterial species in cranial orifices of dogs in Makurdi

Ten (10) bacterial species were isolated (Table 1). *Staphylococcus* spp., 88 (35.48%), was the most frequent. Others were *E. coli;* 54 (21.77%), *S. aureus;* 41 (16.53%), *Klebsiella* spp.; 27 (10.89%), *Streptococcus* spp.; 24 (9.68%), *P. multocida;* 4 (1.61%), *E. faecalis;* 3 (1.21%), *Salmonella* spp.; 3 (1.21%), *Micrococcus* spp.; 2 (0.81%), and *Neisseria* spp.; 2 (0.81%). There was a significant association of bacterial species with dogs ($\chi^2 = 119.495; df = 9; P = 0$).

The oral orifice had the highest bacterial occurrence of 71 (28.63%). All bacterial species isolated were found in the mouths, except *Micrococcus* spp. This was followed by bacteria from the auditory orifices, 60 (24.19%), ocular orifices, 59 (23.79%), and nasal orifices, 58 (23.39%). However, no significant relationship between bacterial occurrence and cranial orifices was observed ($\chi^2 = 34.6; df = 4; P = 0.15$).

Table 1: Distribution of Isolated Bacteria from the Cranial Orifices of Dogs in Makurdi

S/no.	Isolate	Cranial Orifice				Total
		Auditory	Nasal	Ocular	Oral	No. (%)
1	*Staphylococcus* sp.	21	21	22	24	88 (35.5)
2	*E. coli*	14	12	11	17	54 (21.8)
3	*S. aureus*	13	9	7	12	41 (16.5)
4	*Klebsiella* sp.	6	8	7	6	27 (10.9)
5	*Streptococcus* sp.	6	5	10	3	24 (9.7)
6	*P. multocida*	0	2	0	2	4 (1.6)
7	*E. faecalis*	0	0	0	3	3 (1.2)
8	*Salmonella* sp.	0	1	0	2	3 (1.2)
9	*Micrococcus* sp.	0	0	2	0	2 (0.8)
10	*Neisseria* sp.	0	0	0	2	2 (0.8)
	Total	60 (24.2)	58 (23.4)	59 (23.8)	71 (28.6)	248 (100)

χ^2 = 34.6; P = 0.151

Distribution of bacterial isolates from the cranial orifices of dog breeds in Makurdi

Table 2 shows bacteria isolated from the cranial orifices of different dog breeds in Makurdi. The Lhasa (Lh) breed had the highest bacterial occurrence of 60 (24.19%), followed by German Shepherds (GS) 50 (20.16%), and mixed breed (Mb) 40 (16.13%). The Eskimo (Ek) had the least bacterial occurrence, 12 (4.84%) observed. Chi-square showed no association between isolates and dog breed *(χ^2 = 57.040; df = 7; P = 0.688)*.

1 **Table 2: Bacterial Isolates from the Cranial Orifices of Dog Breeds**

	Breed of Dog								
Isolate	**Ca** (%)	**Ek** (%)	**Gs** (%)	**Hm** (%)	**Lh** (%)	**Mb** (%)	**Mg** (%)	**Pm** (%)	**Total** (%)
Staphylococcus sp.	14 (5.7)	4 (1.6)	18 (5.2)	4 (1.6)	17 (6.9)	15 (6.1)	8 (3.2)	8 (3.2)	88 (35.5)
E. coli	8 (3.2)	1 (0.4)	12 (4.8)	4 (1.6)	11 (4.4)	10 (4.0)	2 (0.8)	6 (2.4)	54 (21.8)
S. aureus	6 (2.4)	3 (1.2)	9 (3.6)	2 (0.8)	9 (3.6)	9 (3.6)	1 (0.4)	2 (0.8)	41 (16.5)
Klebsiella sp.	3 (1.2)	1 (0.4)	5 (2.0)	1 (0.4)	9 (3.6)	5 (2.0)	2 (0.8)	1 (0.4)	27 (10.9)
Streptococcus sp.	4 (1.6)	2 (0.8)	3 (1.2)	-	12 (4.8)	-	2 (0.8)	1 (0.4)	24 (9.7)
P. multocida	1 (0.4)	-	-	-	1 (0.4)	1 (0.4)	1 (0.4)	-	4 (1.6)
E. faecalis	1 (0.4)	1 (0.4)	-	1 (0.4)	-	-	-	-	3 (1.2)
Salmonella sp.	1 (0.4)		1 (0.4)	-	1 (0.4)	-	-	-	3 (1.2)
Micrococcus sp.	1 (0.4)	0 (0)	1 (0.4)	-	-	-	-	-	2 (0.8)
Neisseria sp.	-	-	1 (0.4)	1 (0.4)	-	-	-	-	2 (0.8)
Total	39 (15.7)	12 (4.8)	50 (21.2)	13 (5.2)	60 (24.2)	40 (16.1)	16 (6.5)	18 (7.3)	248 (100)

$\chi^2 = 57.040; P = 0.688$

Key: Ca = Caucasian, Ek = Eskimo, Gs = German Shepherd, Hm = Hamid, Lh = Lhasa, Mb = Mixed breed, Mg = Mongrel, Pm = Pomeranian.

Bacterial isolates from the cranial orifices of dogs by weight

Table 3 shows the distribution of bacterial isolates from the cranial orifices of dogs in Makurdi based on the weight. Dogs that were 0 - 12 kg had the highest bacterial occurrence of 84 (33.87%), followed by 13 – 24 kg; 80 (32.26%), 25 – 36 kg; 43 (17.34%), and 37 – 48 kg; 41 (16.53%). The occurrence decreased with increase in weight. No significant relationship between bacteria and weight was observed *(χ^2 = 27.230; df = 3; P = 0.451)*.

Table 3: Frequency of Bacteria Isolated from Cranial Orifices of Dogs by Weight

	Weight (kg)				
Isolate	0 – 12	13 – 24	25 – 36	37 – 48	**Total** (%)
Staphylococcus sp.	31	27	14	16	88 (35.5)
E. coli	20	18	9	7	54 (21.8)
S. aureus	13	16	8	4	41 (16.5)
Klebsiella sp.	8	9	5	5	27 (10.9)
Streptococcus sp.	12	6	3	3	24 (9.7)
P. multocida	-	1	1	2	4 (1.6)
E. faecalis	-	-	1	2	3 (1.2)
Salmonella sp.	-	1	1	1	3 (1.2)
Micrococcus sp.	-	2	-	-	2 (0.8)
Neisseria sp.	-	-	1	1	2 (0.8)
Total (%)	**84** (33.9)	**80** (32.3)	**43** (17.3)	**41** (16.5)	**248** (100)

χ^2 = 27.230; P = 0.451

Distribution of bacterial isolates based on age

The distribution of bacterial isolates based on the age of dogs is presented in Table 4. Dogs that were 0 - 12 months old had the highest bacterial occurrence of 114 (45.97%). This was followed by dogs aged between 37 - 56 months; 57 (22.98%), 13 – 24 months 48 (19.35%), and 25 – 36 months 29 (11.69%). No significant relationship between bacteria and age of dogs was observed in the study *(χ^2 = 38.307; df = 3; P = 0.073)*.

Table 4: Frequency of Bacterial Isolates by Age

	Age (months)				
Isolate	0 – 12	13 - 24	25 – 36	37 - 56	**Total** (%)
Staphylococcus sp.	45	27	14	16	88 (35.48)
E. coli	26	18	9	7	54 (21.77)
S. aureus	15	16	8	4	41 (16.53)
Klebsiella sp.	12	9	5	5	27 (10.89)
Streptococcus sp.	16	6	3	3	24 (9.68)
P. multocida	-	1	1	2	4 (1.61)
E. faecalis	-	-	1	2	3 (1.21)
Salmonella sp.	-	1	1	1	3 (1.21)
Micrococcus sp.	-	2	-	-	2 (0.81)
Neisseria sp.	-	-	1	1	2 (0.81)
Total (%)	**114** (45.97)	**48** (19.35)	**29** (11.69)	**57** (22.98)	**248 (100)**

χ^2 = 38.307; P = 0.073

Distribution of bacterial isolates based on sex of dogs

The distribution of bacterial isolates from the cranial orifices of dogs in Makurdi based on sex is presented in Table 5. The result showed that male dogs had higher bacterial occurrence, 146 (58.87%), than females, at 102 (41.13%). There was, again, no significant relationship between bacterial occurrence and sex of the dogs in the study area *(χ^2 = 12.565; df = 1; P = 0.183)*.

Table 5: Frequency of Bacterial Isolates by Sex

Isolates	Sex		Total
	Male	Bitches	
Staphylococcus spp.	50	38	88 (35.48)
E. coli	31	23	54 (21.77)
S. aureus	28	13	41 (16.53)
Klebsiella spp.	18	9	27 (10.89)
Streptococcus spp.	14	10	24 (9.68)
P. multocida	2	2	4 (1.61)
E. faecalis	-	3	3 (1.21)
Salmonella spp.	-	3	3 (1.21)
Micrococcus spp.	1	1	2 (0.81)
Neisseria spp.	2	-	2 (0.81)
Total (%)	**146** (58.87)	**102** (41.13)	**248** (100)

$\chi^2 = 12.565; P = 0.183$

Discussion

The cranial orifices of healthy dogs have diverse bacterial species, as shown in this study. The oral cavity was dominated by *Staphylococcus* spp., followed by several other bacterial species. Abundance of *Staphylococcus* spp. could be because, as normal flora, it can be easily picked up from the environment and introduced into various orifices [13]. The isolation of *E. faecalis*, *E. coli*, and *Klebsiella* from the oral cavity of bitches might be due to the maternal grooming of puppies and coprophagy [14]. One study linked the presence of some of these bacteria to the age, sex, and breed of dog [15]. Another study linked the bacteria from the oral cavities of dogs to poor oral hygiene practices by owners [16]. In this study, oral hygiene is seldom practiced locally on dogs by their owners. This could also have led to the proliferation of the isolated bacteria in the buccal cavities of these dogs.

The bacteria encountered in this study are also found in earlier investigations on the bacteria of the oral cavity of dogs. [17, 18 and 13] reported the isolation of aerobic bacterial species such as *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Streptococcus* spp., *Klebsiella* spp., and *P. multocida,* which is consistent with the findings in this study but varies in the isolation of *Bacillus, Corynebacterium*, *Listeria*, *Moraxella,* and *Proteus* as reported by [17]. The rate of isolation observed in this study is higher than that reported by [13]. Moreover, the frequency of bacteria isolated in this study differs from other reports. For instance, *E. coli* was the most predominant bacterial species reported by [17] and [13]. *Bacillus* spp. was the predominant bacterium reported by [18].

The differences in the observed frequency might be due to the management system of dogs. Out of the 25 dogs sampled in this study, only two dogs were under restricted intensive management. The rest were managed extensively. This meant that the dogs, roaming freely, had a greater opportunity to pick up diverse bacteria from the environment.

While [18] showed that there is no breed, age, sex, or management system variation of bacteria in the oral cavities of apparently healthy dogs, microbiota is influenced by management systems regarding food consumed, the environment, and the type of water drunk. Factors such as scavenging play a part in the bacteria found in the oral cavity.

[14] identified a rich and diverse bacterial species through mass spectrometry, much more than was found in this study. [19, 20 and 21] reported the isolation of bacterial species such as *Staphylococcus* spp., *E. coli, Streptococcus, Staphylococcus aureus,* and *Klebsiella* spp. using Next Generation Sequencing, which readily identifies a broad range of both culturable and non-culturable bacterial species. This is consistent with the findings in this study but varies in the isolation of *Actinobacter, Pseudomonas, Proteus,* and *Citrobacter*. The scope of identification is deeper than the present culture-based identification.

The nasal cavities were also laden with diverse bacterial species. Dogs frequently lick their noses, a practice that helps to keep them wet and moist. This invariably transfers bacteria from the buccal cavities to their noses, which may then find entrance into the nostrils. Moreover, bacteria from the nostrils may have been from

The most frequently isolated bacteria reported by [21] were *E. coli* (18.5%), while this study found *Staphylococcus* spp. (8.5%). The isolation rates observed by [22] are very similar to those of this study in the sense that *Salmonella* spp., *Streptococcus* spp., *Staphylococcus* spp., *E. coli,* and *Klebsiella* spp. were also reported in their work.

Ocular surfaces were found to contain diverse aerobic bacterial species. Previous reports by [23] and [24] also align with the isolation pattern of this study. While [25] also reported the isolation of *Staphylococcus* spp. *Micrococcus* spp. and *Streptococcus* spp. the variation is that they isolated *Trueperella*, *Bacillus*, *Aeromonas*, *Pseudomonas,* and *Neisseria* spp.

The isolation of bacteria from the ears of healthy dogs revealed a higher percentage of *Staphylococcus* spp., followed by *E. coli*, *S. aureus*, *Streptococcus* spp., and *Klebsiella* spp., which supports the findings of previous reports [26]. However, they also isolated *Bacillus*. There is a similarity of the auditory canal isolates by [27] and this study. The difference, however, may be due to the different methods used for analyses of samples, as they also used Next Generation Sequencing.

As puppy numbers in this study were higher than in the other ages, this might account for the higher bacterial occurrence in them. Moreover, puppies have a relatively underdeveloped immune system and lack the grooming skills of older dogs [28, 29].

The isolation of greater bacteria from some breeds of dogs was also probably because of the higher sample sizes

of these breeds compared with others, which were maller in number.
[30] suggests that gender and weight have effect on the bacteria isolated from dogs, in this study, gender and weight of dogs did not show significant relationship with the occurrence and prevalence of bacteria found in the cranial orifices. This could be because as dogs sampled were all within the weight ranges expected for their ages. However, the observed trend was that with increasing weight, signifying growth and maturity, both in size and immune development. This study shows that most of the bacterial species isolated are pathogenic to humans. *P. multocida* causes abscesses, cellulitis, severe purulent wounds, and lymphangitis when found in numerous bite wounds [30]. The genus *Neisseria* has been identified as the primary cause of septicemia in patients bitten by dogs [31].
Dogs transmit viral and bacterial diseases to humans through bites, scratches, fur, saliva, petting, and grooming, introducing bacteria such as *Staphylococcus* spp., *Streptococcus* spp., *Salmonella* spp., *Neisseria* spp., *E. coli,* which were all isolated in this study.
Dogs' coughs and sneezes have the potential to transmit respiratory bacteria such as *Streptococcus* spp. leading to respiratory symptoms like sneezing, runny noses, and itchy eyes. Individuals with weekend immune systems or immune-compromised individuals are at higher risk of contracting these pathogenic bacteria.

Conclusion

Various bacteria were isolated from the cranial orifices of apparently healthy dogs. *Staphylococcus* spp. had the highest occurrence. Although some bacterial species isolated are not naturally pathogenic, they may become opportunistic pathogens in situations such as immune suppression. The high rates of bacterial isolation recorded in this study suggest that while dogs were not sick, nor did they show symptoms of disease, they could be significant reservoirs of multiple bacterial pathogens of humans.

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