

ORIGINAL ARTICLE OPEN ACCESS

Dogs

Antibiotic Susceptibility of Zoonotic Bacteria Isolated From Oral Cavities of Indigenous Dogs From Semi-Urban Areas in Uganda

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Received: 14 February 2024 | **Revised:** 7 November 2024 | **Accepted:** 29 November 2024

Funding: The authors received no specific funding for this work.

Keywords: dog bites | oral cavities | susceptibility | zoonotic bacteria

ABSTRACT

Dog bites are a source of zoonotic infections to humans, such as pasteurellosis and meningitis. Zoonotic bacterial identification and their antibiotic susceptibility assessment are key towards the successful management of such infections. This study isolated and identified zoonotic bacterial species from the oral cavities of indigenous dogs and also determined their antibiotic susceptibility profile. Oral swab samples collected from 54 indigenous dogs (domestic [36] and stray [18]) were cultured on agar media, and then on selective-differential media. The colony morphology and conventional biochemical tests were used to identify the bacterial isolates. Antibiotic susceptibility was evaluated using the Kirby–Bauer disk diffusion method. A total of 232 bacterial isolates were obtained, from which 29 bacteria species (18 Gram-negative and 11 Gram-positive) belonging to 19 genera and 13 families were identified. Aerobic and anaerobic bacteria species constituted 69% and 31%, respectively. The most prevalent bacteria species was *Staphylococcus aureus* (10.8%) followed by *Escherichia coli* (9.5%), while *Yersinia enterocolitica* (0.4%) was the least common. Most bacteria species belonged to family Enterobacteriaceae (11) followed by Vibrionaceae (04). All the sampled dogs had a number of multi-drug-resistant superbugs in their oral cavities. However, all the tested bacterial isolates were only susceptible to imipenem and chloramphenicol. All the identified bacteria species were resistant to metronidazole, amoxicillin-clavulanic acid and ceftazidime. Therefore, antibiotics such as imipenem and chloramphenicol could be of better choice for managing dog bite infections compared to the antibiotics recommended by Uganda Clinical Guidelines, such as metronidazole and amoxicillin-clavulanic acid.

1 | Introduction

Dogs are among the tamed intelligent animals that live with man worldwide. They serve various purposes such as cultural, economic, social, protection, forensic and companionship (Bata

et al. 2020). Human interactions with dogs, either casually or tactically such as in petting and territorial defence, result in dog bites, since some dogs tend to retain wild instincts (Owczarczak-Garstecka et al. 2019). Dog bites constitute the highest percentage of emergency hospital visits as compared to canine bites globally

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(Goldstein et al. 2018; Morzycki, Simpson, and Williams 2019; Razali et al. 2020). In developing countries of Asia and Africa, dog bites constitute over 95% of animal bite cases (Kisaka et al. 2021). In Uganda, dog bites are a public health concern, with an annual prevalence of over 14,865 bites reported between 2015 and 2020 (Akusekera, Namayanja, and Okello 2021). According to Wangoda et al. (2019), there are more dog bites caused by stray dogs than domestic dogs in semi-urban areas of which such dogs are majorly indigenous.

Dog bites are reported as an important source of infections to humans (Hampson et al. 2015) such as pasteurella, meningitis, salmonella infections (Ghasemzadeh and Namaz 2015), among others. The dog bite wounds are commonly infected through microbial contamination in addition to other complex effects (Mouro, Vilela, and Niza 2010) such as tetanus and rabies (Griego et al. 1995). Up to 18% of dog bite wounds are infected with the dog's oral flora, and such infections are referred to as polymicrobial (Razali et al. 2020), since the dog's oral cavity contains numerous bacteria and other microbes. Studies have reported that several bacteria species such as *Pasteurella species* (Damborg et al. 2016), *Staphylococcus aureus*, *Escherichia coli* (Meyer et al., 2012; Kaspar et al. 2019) and *Clostridium perfringens* (Griego et al. 1995) are common zoonotic pathogens to humans from dog's oral flora cavities. This implies that the oral flora cavity of the biting dog is a source of bacteria species (Talan et al. 1999; Rothe, Tsokos, and Handrick 2015) that could infect the dog bite wound. Meyers et al. (2008) stated that there is always need for veterinary care such as vaccination of dogs, so as to minimize bacterial pathogen drift to man via dog bites. Unfortunately, according to Akusekera, Namayanja, and Okello (2021), only 10% of dogs in Uganda are vaccinated that puts dog bite victims at risk on acquiring infections due to dog bites.

It has also been reported that animal bites such as dog bites are significant sources of resistant bacterial pathogenic strains to humans (Yaovi et al. 2022). This means that the bacteriology and susceptibility tests on the pathogens from such bites are paramount to guide clinicians on the choice of antibiotic therapy (Kisaka et al. 2022) to manage those bites. Therefore, this study focussed on isolation, identification and antibiotic susceptibility testing of zoonotic bacteria pathogens from the oral cavities of indigenous (domestic-chained, domestic-free and stray) dogs. The results may act as a reference point for antibiotics choice in the clinical management of dog bite wound infections.

2 | Materials and Methods

2.1 | Study Designs and Study Areas

A cross-sectional study was used to collect information from 27 dog owners between June 2022 and January 2023. Domesticated dogs were purposively selected using area local council members who knew the homes with domestic-chained and/or domestic-free dogs. After dog owners consented to participate in the study, they were required to give information on the dogs' demography, vaccination status, diet, treatment and management of bite wounds when dog bites occur. The stray dogs were captured and samples were collected with the help of experienced dog handlers. In this study, a domestic-chained dog is a dog owned

in a particular homestead, kept on a chain during day time and released at night time but it is not allowed to move out of the compound of a homestead. The domestic-free dog is one owned and kept in a homestead but it is free to move out of the compound of a homestead. A stray dog is one that roams in and around an area and its homestead of ownership is not known.

The dogs were sampled from semi-urban areas of the western, central and eastern regions of Uganda, which were purposively selected based on the high prevalence of dog bites (Wangoda et al. 2019). The specific areas selected were as follows: Ishaka-Bushenyi municipality (0.5468°S, 30.1387°E–0.5424°S, 30.1965°E) in the western region; Kawempe division in Kampala (0.3802°N, 32.5571°E) in the central region and Mafubira division, Jinja city (0.4824°N, 33.2132°E) in the eastern region.

2.2 | Sample Size Determination

The sample size was determined using the formula described by Almeda, Capistrano, and Sarte (2010):

$$n = \frac{Nz^2}{z^2 + 4Ne^2}, \quad (1)$$

where n = the sample size, z = the z -score at 90% confidence level, e = marginal error of 10% and N = the population of dogs in Uganda. The animal census carried out in 2008 reported approximately 1.6 million dogs in Uganda (Masiira et al. 2018). Using Equation (1), the sample size calculated was 68 dogs. There are four regions in Uganda, and there are three categories of dogs (domestic-chained, domestic-free and stray) studied. Therefore, approximately 18 dogs per region comprising 6 dogs per category were sampled. This means that the dog sample size for the three regions studied was 54 dogs consisting of 36 domestic and 18 stray dogs.

2.3 | Swab Sample Collection, Handling and Bacteria Culturing

The oral cavity samples were collected in triplicates using sterile swabs by rubbing the tongue, gum, palate, and teeth of each dog. The samples were transported in an ice box and stored at 4°C before analysis. The swab samples were cultured for bacterial growth as described by Nagy, Boyanova, and Justesen (2018) and Razali et al. (2020) with slight modification. Briefly, the swab samples were inoculated on chocolate agar and blood agar supplemented with 5% defibrinated sheep blood. The plates were, respectively, incubated, anaerobically in a 5% CO₂ atmospheric chamber containing HiAnaero Gas Pack in HiMedia Gas PAK and aerobically at 37°C for 48–72 h.

2.4 | Bacterial Isolation and Identification

Isolation and identification of the bacteria species were carried out as described by Nagy, Boyanova, and Justesen (2018) with slight modifications as per Razali et al. (2020). The incubated culture plates were examined for presumptive identification of the bacteria based on colony morphologies formed on either

chocolate and/or blood agar before purification on differential media. The presumptive colonies were sub-cultured on blood agar, MacConkey agar, mannitol salt agar, nutrient agar, thiosulfate citrate bile salts, xylose lysine deoxycholate agar and eosine methylene blue agar, and then they were incubated under the respective aeration conditions at 37°C for 48–72 h. Thereafter, phenotypic identification of the bacteria species was done based on the morphology of the colonies, type of haemolysis and nature of lactose fermentation. This was followed by performing Gram staining microscopy and conventional biochemical tests of catalase, oxidase, coagulase, indole, methyl red–Voges–Proskauer (MR–VP), urease, citrate, hydrogen sulphide producing, nitrate reduction, lactose fermentation and lysine decarboxylation. The absence of bacteria growth in specific media was also considered to draw the inference. The most common bacterial species identified from all the study regions were selected for antibiotic susceptibility tests.

2.5 | Antibiotic Susceptibility Tests by Kirby–Bauer Disk Diffusion Method

Thirteen commonly prescribed antibiotics for animal bites according to Uganda Clinical Guidelines (UCG) (2016), azithromycin (AZM, 15 µg), chloramphenicol (C, 30 µg), kanamycin (K, 30 µg), cefotaxime (CTX, 30 µg), ceftriaxone (CTR, 30 µg), imipenem (IMP, 10 µg), amoxicillin-clavulanic acid (AMC, 20 µg), ceftazidime (CAZ, 30 µg), tetracycline (TE, 30 µg), sulphamethoxazole-trimethoprim (SXT, 23 µg), ciprofloxacin (CIP, 5 µg), gentamycin (GEN, 10 µg) and metronidazole (MTZ, 10 µg), were used for antibacterial susceptibility testing following the Clinical Laboratory Standard Institute (CLSI) guidelines (CLSI 2018). Two discrete purified colonies were emulsified in 2 mL of 0.85% w/v normal saline to obtain a bacterial suspension with turbidity equivalent to 0.5 McFarland standards (Messele et al. 2017). The bacterial suspension was spread uniformly on the surface of Mueller–Hinton agar (HIMEDIA, India) using a sterile swab while rotating the plate at approximately 60° thrice. The inoculated plates were left to dry at room temperature for approximately 15 min. Unlike other test bacteria, *Pasteurella multocida* isolates were inoculated on Muller–Hinton agar supplemented with 5% defibrinated sheep blood. Subsequently, selected antibiotic discs (Oxoid, UK) were added to the surface of the inoculated agar plates using sterile forceps. The plates were later incubated aerobically or anaerobically based on the aeration conditions needed by the inoculated bacteria (CLSI 2018). After incubation, the growth inhibition zone diameters were measured using a ruler in millimetres, and thereafter interpreted as susceptible, intermediate or resistant (CLSI 2020). All tests were performed in triplicates.

Media performance tests were performed on freshly prepared media. Briefly, the prepared sterile agar plates were inoculated with standard American Type Culture Collection (ATCC) bacteria strains of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 for quality control of all media used. Also, the subculture of *Pseudomonas aeruginosa* ATCC 27853 on Simmon's citrate slants was incubated in a chamber that contained an anaerobic GasPAK as a control (Nagy, Boyanova, and Justesen 2018).

2.6 | Data Analysis

Descriptive data were presented as frequencies and percentages. Numerical data were tabulated using MS-Excel for Windows (MS Corporation, USA). The zones of inhibition were used to assess antibiotic susceptibility patterns.

3 | Results

3.1 | Demographic Characteristics of Indigenous Dogs Studied

The demographic characteristics of 54 indigenous domestic and stray dogs from semi-urban areas in Uganda, composed of 61.1% males and 38.9% females, are presented in Table 1. The average age range of most domestic dogs was between 1 and 2 years (41.7%).

The study revealed that the common purpose of owning and domesticating dogs was for security (83.3%). Thus, male dogs were preferred to female dogs. Most domestic dogs were fed on the same food as that prepared for consumption in the homes of dog owners (69.4%), followed by restaurant leftovers (16.7%), while 13.9% feed on their own, especially the domestic-free dogs. A big proportion (44.4%) of domestic dogs were treated annually, while 16.7% received no treatment. To maintain hygiene, some shelters were cleaned regularly by sweeping (30.5%), disinfected (11.1%) and some dogs were washed (8.3%). It was observed that 50% of domestic dogs were vaccinated once a year, while the rest were either not vaccinated at all (30.6%) or were vaccinated massively during government programmes (19.4). Very few dog owners were aware of dog bite management and could attend to a victim by either rushing him/her to the nearby clinic (18.5%) or applying medicinal plants from herbalists (14.8%). Dog owners (66.7%) had no idea about dog bite management. The profile of stray dogs was not fully described in Table 1 because of their unknown living status. The 18 stray dogs included in the study were generally obtained around garbage collection sites and open playgrounds.

3.2 | Bacteria Species Isolated From the Oral Cavities of the Dogs

A total of 232 cultivable bacterial isolates were obtained from all the oral swabs cultured. On identification, 29 bacteria species belonging to 19 genera and 13 families were obtained (Table 2).

Bacteria species from the family Enterobacteriaceae dominated, followed by those from the Vibrionaceae family (Figure 1).

The study also showed that 69% (20/29) of the bacteria isolated were aerobic, while 31% (9/29) were anaerobic. Among the aerobic bacteria, 65.0% were Gram-negative and 35.0% were Gram-positive (Table 2). The more prevalent aerobic bacteria species were *S. aureus* (10.8%), followed by *E. coli* (9.5%), while the least prevalent was *Y. enterocolitica* (0.4%) of the total isolates.

Gram-negative (55.6%) anaerobic bacteria species were the most common as compared to Gram-positive (44.4%) anaerobic

TABLE 1 | Demographic profile of domestic and stray indigenous dogs and dog bite management by dog owners from semi-urban area in Uganda.

Feature		Description	Frequency	Percentage
Sex		Male	33	61.1
		Female	21	38.9
Age	Domestic	< 1 year	07	19.4
		1–2 years	15	41.7
		2–5 years	11	30.6
		≥ 5 years	03	8.3
		NA	NA	NA
Purpose	Domestic	Security	30	83.3
		Pet (company)	04	11.1
		Business	02	5.5
	Stray	NA	NA	NA
Shelter	Domestic	Kernel	03	8.3
		Chained in sheds	15	41.7
		Free	18	50.0
	Stray	NA	NA	NA
Feeding	Domestic	Any food prepared in owner's home	25	69.4
		Leftovers got outside owner's home	06	16.7
		Left to feed on their own	05	13.9
	Stray	NA	NA	NA
Hygiene	Domestic	Dogs bathed regularly	03	8.3
		Regular disinfecting on the dog shelter	04	11.1
		Regular sweeping in the dog shelter	11	30.6
		No hygiene measure taken	18	50.0
	Stray	NA	NA	NA
Frequency of treatment	Domestic	Treated when young only	06	16.7
		Treated annually	16	44.4
		Treated when sick only	06	16.7
		Rarely treated	02	5.5
		Not treated at all	06	16.7
	Stray	NA	NA	NA
Frequency of vaccination	Domestic	Vaccinated annually	18	50.0
		Vaccinated during massive programmes	07	19.4
		Not vaccinated at all	11	30.6
	Stray	NA	NA	NA
Dog bite management by dog owners		Clean wound with water and takes the victim to the nearby clinic	05	18.5
		Apply medicinal plants	04	14.8
		No idea at all	18	66.7

Note: For domestic dogs ($n = 36$, 66.7%), for stray dogs ($n = 18$, 33.3%), total number of dogs studied was 54, total number of dog owners interviewed ($n = 27$). Abbreviation: NA = not applicable.

bacteria species. *Clostridium* species (03) were more prevalent, followed by *Fusobacterium* species (02), while *Veillonella* species (01) were the least prevalent anaerobic bacteria (Table 2). Out of the 19 genera identified, *Vibrio* (13.8%) was the prevalent genus, followed by *Clostridium* genus (10.3%) (Table 2).

3.3 | Bacterial Susceptibility to Conventional Antibiotics

Thirteen common bacteria species identified from the oral cavities of domestic and stray indigenous dogs were subjected to

TABLE 2 | Bacteria species isolated from the oral cavities of domestic and stray indigenous dogs from semi-urban areas in Uganda.

Bacteria type	Bacteria species	No. of isolates	Isolates (%)	Bacteria species	No. of isolates	Isolates (%)
Aerobic bacteria	Gram-negative (13, 65%)			Gram-positive (07, 35%)		
	<i>Citrobacter freundii</i> ^{WEC}	11	4.7	<i>Bacillus subtilis</i>	03	1.3
	<i>Citrobacter koseri</i>	03	1.3	<i>Enterococcus faecalis</i> ^{WEC}	13	5.6
	<i>Escherichia coli</i> ^{WEC}	22	9.5	<i>Corynebacterium diphtheriae</i>	06	2.6
	<i>Klebsiella oxytoca</i>	04	1.7	<i>Nocardia asteroides</i>	03	1.3
	<i>Klebsiella pneumoniae</i> ^{WEC}	09	3.9	<i>Staphylococcus aureus</i> ^{WEC}	25	10.8
	<i>Pasteurella multocida</i> ^{WEC}	15	6.5	<i>Streptococcus canis</i>	03	1.3
	<i>Proteus mirabilis</i> ^{WEC}	16	6.9	<i>Streptococcus mutans</i> ^{WEC}	10	4.3
	<i>Proteus vulgaris</i>	05	2.2			
	<i>Vibrio alginolyticus</i>	02	0.9			
	<i>Vibrio cholerae</i> ^{WEC}	12	5.2			
	<i>Vibrio parahaemolyticus</i> ^{WEC}	12	5.2			
	<i>Vibrio vulnificus</i>	04	1.7			
	<i>Yersinia enterocolitica</i>	01	0.4			
Anaerobic bacteria	Gram-negative (05, 55.6%)			Gram-positive (04, 44.4%)		
	<i>Bacteroides fragilis</i> ^{WEC}	11	4.7	<i>Clostridium difficile</i>	03	1.3
	<i>Fusobacterium necrophorum</i>	03	1.3	<i>Clostridium perfringens</i> ^{WEC}	08	3.5
	<i>Fusobacterium nucleatum</i>	03	1.3	<i>Clostridium septicum</i>	02	0.9
	<i>Prevotella nigrescens</i>	09	3.9	<i>Peptostreptococcus asaccharolyticus</i> ^{WEC}	09	3.9
	<i>Veillonella parvula</i>	05	2.2			

Note: WEC = Bacterium isolated from oral dog swabs from western, eastern, and central regions. Total number of bacterial isolates ($n = 232$), aerobic bacteria species ($n = 20$, 68.9%) and anaerobic bacteria species ($n = 9$, 31.1%).

antibiotic susceptibility tests against 13 antibiotics commonly prescribed for the treatment of animal bites. The antibiotic susceptibility results revealed that none of the selected bacterial isolates was susceptible to all the 13 antibiotics used (Table 3).

The order of descending susceptibility to antibiotics was: *B. fragilis* = *P. mirabilis* > *P. multocida* = *E. coli* > *S. aureus* > *V. parahaemolyticus* > *S. mutans* > *C. perfringens* > *V. cholera* = *E. faecalis* = *K. pneumoniae* = *C. freundii* > *P. asaccharolyticus* (Figure 2). However, all the selected bacterial isolates were 100% susceptible to chloramphenicol and imipenem, followed by azithromycin and tetracycline (76.9%), then kanamycin (61.5%) and trimethoprim (53.8%).

Similarly, all the selected bacterial isolates were 100% resistant to metronidazole, amoxicillin and ceftazidime, followed by ceftriaxone, cefotaxime and ciprofloxacin (69.2%) (Figure 3).

4 | Discussion

The findings revealed that the most dogs studied were males than females that are domesticated majorly for security, which concur with the findings of Awoyomi and Ojo (2014). The dogs are mostly fed any food as that prepared in dog owners' home, and their hygienic conditions are bad, although they are at least treated or vaccinated annually. According to Oh et al. (2015), the nature of food consumed, age and hygienic conditions are relevant to the health of dogs. For example, the periodontal health of dogs and the oral microbiome depends on the diet (Isaiah, Hoffmann, and Kelley 2017; Kardjadj and Ben-Mahdi 2019). Meyers et al. (2008) reported that the veterinary care of dogs is instrumental to sustaining their good health. Poor health interventions put dogs at risk of being invaded by pathogens that affect their immune system. Therefore, diet, living conditions and limited veterinary care have put the majority of the dogs in the study areas at health risk of being invaded by microbes in their oral

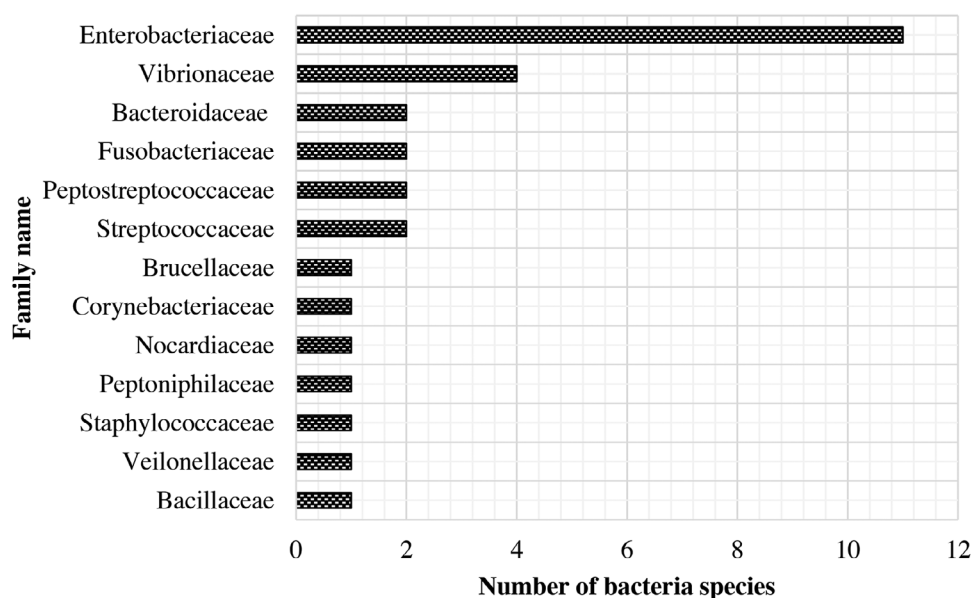


FIGURE 1 | Families of the bacteria species isolated from the oral cavities of domestic and stray dogs from semi-urban areas in Uganda.

TABLE 3 | Antibiotic susceptibility profile of the selected common bacteria species isolated from oral cavities of domestic and stray dogs from semi-urban areas in Uganda by disk diffusion method.

Commonly identified bacteria species	Antibiotics used and mean zones of inhibition (mm), <i>n</i> = 3												
	AZM	C	K	CTX	CTR	IPM	AMC	CAZ	TE	STX	CIP	GEN	MTZ
<i>B. fragilis</i>	12	30*	19*	27*	30*	32*	0	0	16*	20*	33*	14*	0
<i>P. mirabilis</i>	20*	26*	20*	24*	27*	29*	0	0	0	24*	30*	15*	0
<i>S. aureus</i>	25*	30*	22*	12	19	44*	0	0	30*	20*	25*	16*	0
<i>E. coli</i>	20*	30*	20*	26*	25*	30*	0	0	25*	22*	23	12	0
<i>V. parahemolyticus</i>	25*	18*	25*	0	10	45*	0	0	24*	35*	34*	12	0
<i>P. multocida</i>	22*	23*	20*	25*	29*	32*	0	0	25*	0	0	14*	0
<i>S. mutans</i>	25*	33*	25*	0	0	29*	0	0	30*	30*	0	0	0
<i>V. cholera</i>	20*	25*	12	0	20	31*	7	7	8	20*	23	12	0
<i>E. faecalis</i>	20*	21*	13	10	13	27*	0	0	24*	0	20	9	0
<i>C. perfringens</i>	16*	34*	17	12	15	34*	0	0	25*	0	11	18*	0
<i>K. pneumoniae</i>	20*	25*	20*	0	0	32*	0	0	0	0	20	12	0
<i>P. asaccharolyticus</i>	14	30*	15	10	13	30*	0	0	25*	0	13	0	0
<i>C. freundii</i>	13	20*	16	8	14	30*	0	0	15*	0	0	17*	0

Note: * indicates susceptible; other values of zones of inhibition in the table show resistance.

Abbreviations: AMC = amoxicillin-clavulanic acid, AZM = azithromycin, C = chloramphenicol, CAZ = ceftazidime, CIP = ciprofloxacin, CTR = ceftriaxone, CTX = cefotaxime, GEN = gentamycin, IMP = imipenem, K = kanamycin, MTZ = metronidazole, SXT = sulphamethoxazole-trimethoprim, TE = tetracycline.

cavities. The stray dogs are also non-hygienic because their living environment and diet are uncontrollable since such dogs reside generally around garbage collection sites and open places like playgrounds. Consequently, in case any dog bite occurs, the oral microbiome could be shed into the bite wound(s) of the victim, and the health of people interacting with such dogs is at risk (Meyer et al. 2012; Yaovi et al. 2022).

Most of the dog owners in the study areas had limited ideas on how to manage dog bites. The findings concur with Kisaka et al.

(2022) who reported that fewer individuals have knowledge on managing dog bites and that some victims apply local materials such as herbs before and or after clinical interventions (Kisaka et al. 2021). This calls for community sensitization by stakeholders to minimize the consequences of dog bites (Mouro, Vilela, and Niza 2010) on the health of the people.

From the findings, each dog had at least one bacterium isolated from its oral cavity. The findings concur with Sturgeon et al. (2013) and Flancman, Singh, and Weese (2018) who reported

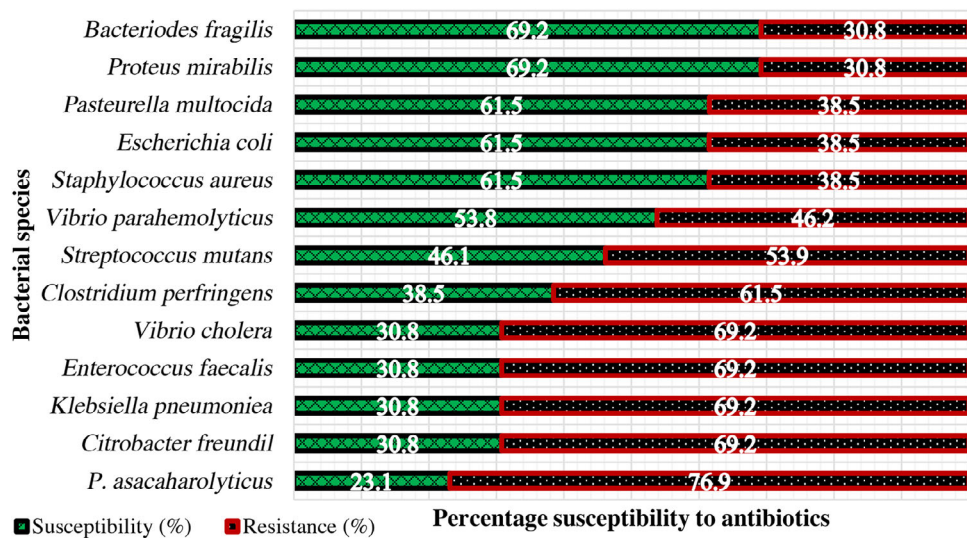


FIGURE 2 | Antibiotic susceptibility pattern of selected bacteria species isolated from the oral cavities of domestic and stray dogs from semi-urban areas in Uganda.

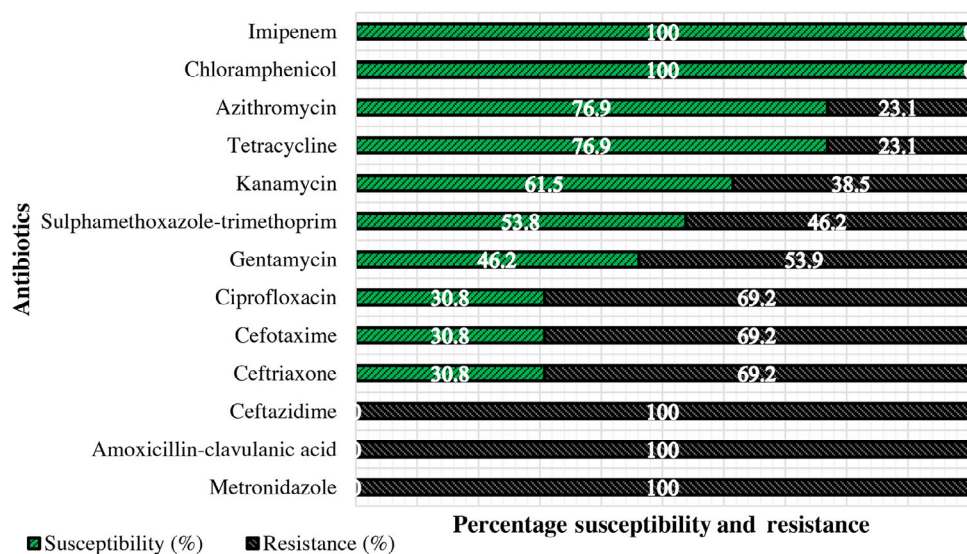


FIGURE 3 | Susceptibility and resistance pattern of selected antibiotics tested against selected bacteria species isolated from the oral cavities of domestic and stray dogs from semi-urban areas in Uganda.

various zoonotic bacteria in the oral cavities of dogs studied. The high prevalence of bacterial isolates of the family Enterobacteriaceae is attributed to their natural presence in the oral cavities, oesophagus (Drzewiecka 2016), and gastrointestinal tracts of animals (Katica et al. 2019). As a result, Enterobacteriaceae spp like *P. mirabilis* and *E. coli* could easily be shed into dog bite wounds and cause health challenges such as wound infections and abscesses, meningitis, septicemia, urinary tract infection, and fatal endotoxemia. The current findings validate prior studies that have reported wound infections from dog bites due to the presence of Enterobacteriaceae bacteria species (Bula-Rudas and Olcott 2018; Katica et al. 2019). Enterobacteriaceae spp are multi-drug-resistant and could result in nosocomial infections (Kaspar et al. 2019). For other bacteria spp of other bacterial families identified, several studies have also reported members of bacterial isolates from oral cavities of dogs belonging to most of these

families (Yaovi et al. 2022; Talan et al. 1999), which concur with the findings of the present study.

Bacteria species in the genera *Vibrio*, *Clostridium*, *Peptostreptococcus*, *Veillonella*, *Yersinia* and *Nocardia* were reported for the first time from the oral cavities of indigenous dog breeds in Uganda. The bacteria species of genus *Vibrio* were more common in the present findings, which contradicts with other past studies of the same kind except Meyers et al. (2008) who isolated Vibrionaceae bacterial isolates in non-infected (02) and infected (02) dog bite wounds in South Africa. The presence of *Vibrio* species could be due to the poor hygienic conditions that most of the domestic (50%) and stray (100%) dogs live in and also their poor diet (Table 1). Razali et al. (2020) reported that factors such as poor hygiene and diet lead to contamination of the dog's oral cavities. The *Vibrio* species identified were associated to both

domestic-free and stray dogs which share living conditions, and the diet of these dogs could even extend to faecal materials which are associated with *Vibrio* species. Tarasiuk and Wainer (2023) reported that East African countries have *Vibrio* species-related diseases, thus, the existence of the causative pathogens is expected in the environment. Tarasiuk and Wainer (2023) further reported that human companion animals and domestic dogs are exposed to *Vibrio* species, while Zheng et al. (2023) reported that *Vibrio* species are the most common pathogens in the microbiome of diseased dogs.

The bacteria species of other genera identified in this study were reported from different dog breeds in African countries such as South Africa (Ruiz et al. 2018), Nigeria (Bata et al. 2020), Algeria (Razali et al. 2020) and in the United States (Griego et al. 1995). The dissimilarity in the bacteria species identified could be caused by variations in the geographical location (Isaiah, Hoffmann, and Kelley 2017), periodontal health, diet, sampling season and area in the oral cavity swabbed (Kardjadj and Ben-Mahdi 2019). It is also worthwhile to report that other than the bacteria species in the genera reported for the first time in the present study, Kisaka et al. (2020) isolated similar bacteria species from infected dog bite wounds in Uganda. These coherent findings support prior research pronouncements that the oral microbiome of the biting animal is released into the bite wounds (Razali et al. 2020), hence responsible for the polymicrobial infections.

The high percentages of Gram-negative bacteria species are in support with the findings of Lau et al. (2016) who reported that such bacteria cause dog bite wound infection because these superbugs are commonly involved in animal bite infections. Gram-negative bacteria species are pathogenic; they cause septicemia and bacteraemia that produce endotoxins, which, on being released into soft body tissue and blood, result in severe shock and fatality (Babalola and Balogun 2013). Oral cavity swab samples of variable breeds of dogs in Nigeria gave Gram-negative bacteria species of *E. coli* (43.2%) followed by *Klebsiella* spp, *Bacillus* spp (8.9%) and the least was *S. aureus* (≈6.0%) (Bata et al. 2020). From Algerian stray dogs, *P. mirabilis* (16.7%) was more prevalent, followed by *P. pneumotropica* (9.8%) and then *Enterobacter* spp (0.6%) (Razali et al. 2020), while from canine pulps in South Africa, *P. multocida* (10.2%) was more common followed by *Moraxella* spp (8.2%) and least were *Enterobacter* and *Proteus* spp (2.0%) (Ruiz et al. 2018). The contrast in the results with the present study could be possibly due to differences in geographical location (Isaiah, Hoffmann, and Kelley 2017) and the buccal part swabbed (Kardjadj and Ben-Mahdi 2019). However, the present findings agree with Kisaka et al. (2022), who reported that *S. aureus* (30.4%) isolated from infected dog bite wounds are most prevalent in Uganda, followed by other similar bacteria species as of those reported in the current study. A total of 93% of the anaerobic and aerobic bacteria species identified in the current study were reported by Talan et al. (1999) who studied infected dog bite wounds in the United States. Therefore, the findings of the present study could also suggest that there is a drift of bacteria species from the oral cavities of dogs into wounds when biting takes place. This is because reports say that dog bite wounds are usually contaminated with the oral microbiome of the biting dog, and can easily cause infections and other complicated effects (Mouro, Vilela, and Niza 2010; Meyer et al., 2012; Yaovi et al. 2022).

Among the identified bacteria species, 13 common bacteria species were selected for antibiotic susceptibility assessment. Determination of the susceptibility of the bacteria from the oral cavities of dogs acts as a reference point for appropriate antibiotic choice in the management of dog bite wound infections. This is due to the fact that the oral microbiome of the biting animal reflects the polymicrobial isolates in the wounds (Razali et al. 2020). Similarly, UCG (2016) recommended that the antibiotic choice for the management of dog bite wounds should have a background of the cultures from the wound and their sensitivity tests. Kisaka et al. (2022) reported that such clinical tests were not routinely practised, putting victims of dog bites at significant risk of the effects of bacterial infections.

All the selected identified bacteria species subjected to antibiotic susceptibility tests were 100% resistant to metronidazole, amoxicillin and ceftazidime. This is because the zones of inhibition measured (Table 3) were in the resistance range for the respective antibiotics used (CLSI 2020). The selected bacterial isolates tested showed resistance to a minimum of four antibiotics; this shows that multi-drug resistance is in play. These findings agree with Awoyomi and Ojo (2014), who tested bacterial isolates from the oral cavities of apparently normal indigenous hunting dogs in Nigeria and reported that each isolate was resistant to at least two antibiotics. On the contrary, all the selected identified bacteria species for susceptibility were 100% susceptible to imipenem and chloramphenicol. This is because the zones of inhibition measured (Table 3) were in the susceptible range for these antibiotics according to CLSI (2020). These findings agree with those of Kisaka et al. (2022) who reported that the bacterial isolates from dog bites were totally sensitive to imipenem and chloramphenicol.

Therefore, from the findings in the current study, antibiotics such as imipenem and chloramphenicol are suggested as the best choices followed by azithromycin and tetracycline for the management of dog bites. However, the suggestion made here contrasts the recommended list of antibiotics for prophylaxis and treatment of dog bite wounds in Uganda (UCG 2016) and the United States (Ellis and Ellis 2014), because other than tetracyclines, the isolates were resistant to the rest of the antibiotics on the list, especially metronidazole and amoxicillin (Figure 3). In a similar study, Kisaka et al. (2022) reported that the bacterial isolates from dog bite wounds were 100% resistant to metronidazole in Uganda. These coherent findings could be a vivid call for UCG to revisit the grounds under which metronidazole is recommended for prophylaxis and treatment of dog bite wounds. This is because the drug could be a dupe of drug resistance and its continued use might put the life of dog bite victims at risk. Similarly, although amoxicillin is one of the first-line choices for prophylaxis and treatment of dog bite wounds (Ellis and Ellis 2014; Al Omran et al. 2020), the isolates used in the current study that could be potential causes of dog bite infections were also 100% resistant to this antibiotic and so is for ceftazidime. The isolates were also 69.2% resistant to ciprofloxacin, cefotaxime and ceftriaxone. The antibiotic resistance displayed by the selected isolates gives a probable risk of resistant bacterial strain drift from dogs to humans due to the casual and tactical associations (Pomba et al. 2017; Owczarczak-Garstecka et al. 2019). Nevertheless, most of the antibiotics tested in this study have shown a greater possibility of therapeutic triumph that they can be of importance

in the management of dog bite wound infections related to the bacteria species isolated from the oral cavities of indigenous dogs from the regions studied.

5 | Conclusion

As one of the first baseline studies of its kind in Uganda, multi-drug-resistant bacteria species from the oral cavities of indigenous dogs have been isolated and identified using conventional methods. Gram-negative aerobic bacteria species were prevalent, and this suggests that polymicrobial wound infection may occur when an individual is bitten by the indigenous dogs in Uganda. The oral cavities of the indigenous dog were colonized by several potential zoonotic bacteria species; hence, tactical or casual interaction could be a potential path for superbug drift from dogs to humans. However, based on the multi-antibiotic resistance pattern observed, the use of antibiotics such as imipenem and chloramphenicol that had shown 100% antibiotics susceptibility could be a milestone in the management of dog bite wounds to counteract the subsequent infections. Nevertheless, the list of antibiotics recommended by UCG for dog bite management should be revisited.

Author Contributions

Paul Mukasa: conceptualization, writing—original draft, methodology, writing—review and editing, data curation, formal analysis. **Patrick Engeu Ogwang:** supervision, validation. **Richard Oriko Owor:** writing—review and editing, supervision, validation. **Julius Bunny Lejju:** supervision. **Hannington Gumisiriza:** conceptualization, writing—review and editing. **Ibrahim Ntulume:** writing—review and editing, methodology. **Christopher Adaku:** writing—review and editing, supervision, validation.

Acknowledgements

We acknowledge all the dog owners and the experienced dog handlers for their willingness to share the information and help to swab the dogs during this study. Great thanks go to the local council members for their guiding information and sacrifices that helped us identify the dog owners. Great appreciations also go to FoS, MUST for the possible financial support that was extended to us for this study.

Ethics Statement

Ethical approvals were obtained from the Mbarara University of Science and Technology Research Ethics Committee (MUST-2021-76), and the Uganda National Council for Science and Technology (UNCST, NS290ES).

Consent

The Local Council authorities of each study area were consulted before enrolling dog owners/“dog men,” who in turn consented before dog swabs were taken.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data generated and analysed during this study were included in this manuscript.

Peer Review

The peer review history for this article is available at <https://publons.com/publon/10.1002/vms3.70169>.

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