Microbiology Research International
Vol. 13(1), pp. 1-6, January 2025
DOI: 10.30918/MRI.131.24.034
ISSN: 2354-2128
Full Length Research Paper

Isolation, characterization and antibiogram of bacteria associated with the mouth of pet dogs in Port Harcourt Metropolis

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Accepted 7 January, 2025

ABSTRACT

This study aimed to isolate and determine the antibiogram of bacteria associated with the mouths of pet dogs in the Port Harcourt Metropolis. A total of five (5) swab samples were aseptically collected from the mouths of pet dogs across different locations in Port Harcourt and cultured on appropriate agar media using standard microbiological techniques. Bacterial isolation and enumeration were conducted using the spread plate method, while antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method. The results showed that total heterotrophic bacterial counts ranged from 1.7×10^5 to 5.9×10^5 CFU/ml. Total coliform counts ranged from 1.0×10^3 to 11.5×10^3 CFU/ml, while total staphylococcal counts ranged from 1.5×10^4 to 3.2×10^4 CFU/ml. A total of twenty-seven (27) bacterial isolates belonging to six (6) genera were identified: *Staphylococcus spp.* (30%), *Bacillus sp.* (22%), *Micrococcus sp.* (4%), *Pseudomonas sp.* (11%), *Klebsiella sp.* (7%), and *Escherichia coli* (19%). Antibiotic susceptibility testing revealed that, among Gram-positive bacteria, *Bacillus sp.* exhibited 50% resistance to ceftazidime and 33% resistance to rifampicin, azithromycin, and ciprofloxacin but was susceptible to cefuroxime (66.7%), levofloxacin (100%), and gentamicin (100%). *Staphylococcus spp.* showed 66.7% resistance to ceftazidime but were susceptible to gentamicin (77.8%), rifampicin (88.9%), and levofloxacin (100%). For Gram-negative bacteria, *E. coli* exhibited 100% resistance to cefuroxime but showed 20% resistance to gentamicin and ciprofloxacin. *Pseudomonas sp.* was 100% resistant to cefuroxime but demonstrated 100% susceptibility to ofloxacin and ciprofloxacin. Given the observed cases of multidrug resistance in this study, it is recommended that pet dog owners administer antibiotics to their pets only under the prescription of a veterinary professional to prevent potential public health outbreaks.

Keywords: Antibiogram, characterization, bacteria, pet dogs, public health.

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INTRODUCTION

In recent years, the ownership of dogs as pets has become increasingly common across various societies worldwide. The companionship, joy, and emotional support provided by dogs have made them cherished members of countless households. However, alongside the benefits of dog ownership come numerous public health challenges that require attention and understanding.

Humans and dogs share a unique bond that dates back thousands of years. This bond transcends mere companionship, as dogs have historically served in various roles, including hunting, herding, and guarding. Today, dogs continue to enrich human lives by serving as therapy animals, guide dogs for the visually impaired and loyal companions (Bradshaw, 2012; Udell and Wynne, 2008).

Despite the many advantages of dog ownership, certain public health challenges cannot be overlooked.

These challenges include zoonotic diseases, allergies, injuries, and the psychological impact of dog-related incidents (Omudu et al., 2010). One of the primary concerns associated with dogs as pets is the transmission of zoonotic diseases. Dogs can act as vectors for various pathogens, including bacteria, viruses, parasites, and fungi, all of which are capable of causing illnesses in humans. For instance, *Campylobacter*, *Salmonella*, *Leptospira*, and *Echinococcus* are among the pathogens that can be transmitted from dogs to humans, posing significant public health risks (Lorusso et al., 2019).

Allergies to dogs are another widespread public health issue, affecting a substantial portion of the population. Dog allergens-primarily found in dander, saliva, and urine can trigger allergic reactions ranging from mild symptoms, such as sneezing and skin rashes, to severe respiratory complications in susceptible individuals (Ownby et al., 2018).

Dog-related injuries, particularly bites, represent another significant public health concern, especially among children. Dog bites can lead to physical injuries, emotional trauma, and, in severe cases, life-threatening infections. Children are especially vulnerable due to their limited understanding of canine behavior and inability to recognize warning signs (Shuler et al., 2020). Beyond the physical health implications, dog-related incidents can also have profound psychological effects on individuals and communities. Traumatic encounters, such as dog bites, can lead to anxiety, phobias, and avoidance behaviors, which negatively impact quality of life and social interactions (Wright et al., 2019).

To prevent public health outbreaks, it is essential to identify and characterize the types of bacteria present in canines and evaluate their susceptibility to common antibiotics. Therefore, the aim of this study is to isolate, characterize, and assess the antibiogram of bacteria associated with the mouths of pet dogs in the Port Harcourt metropolis.

MATERIALS AND METHODS

Study Area

The study was conducted in selected locations within Port Harcourt City, Rivers State, Nigeria. Samples were collected from five different locations by rotating a moist swab stick inside the mouths of different pet dogs (Table 1).

Table 1. Sample code and locations.

CODE	GPS CO-ORDINATE	LOCATION
NTR	Lat 4.8604010 Long 6.9690340	NTA Road
DL	Lat 4.7935970 Long 6.997827	D-Line
M3	4^{o}48’18” N 6^{o}59’29” E	Mile 3
EI	4^{o}47’37” N 6^{o}59’3” E	Eagle Island
Sr	4^{o}52’0” N 6^{o}58’28” E	Sars road

Sample collection

A total of five (5) samples were obtained, one from each dog, by rotating a moist swab stick in the mouth of each pet dog. The samples were immediately transported in an ice-packed container to the Microbiology Laboratory at Rivers State University for microbiological analysis.

Enumeration of Total Heterotrophic Bacteria (THB)

The total heterotrophic bacterial population was determined from the samples. Each sterile swab stick used for collection was transferred into 3 mL of sterile normal saline separately to obtain a 10^{-1} dilution. The samples were then subjected to ten-fold serial dilution up to a maximum dilution of 10^{-4}. From each dilution, a 0.1 mL aliquot was inoculated separately onto Nutrient Agar (NA) and Eosin Methylene Blue Agar (EMB). After an incubation period of 48 hours, the colonies that grew on NA and EMB were counted. The colony counts, expressed as colony-forming units per milliliter (CFU/mL), were used to calculate the total heterotrophic bacterial population (Prescott et al., 2011).

$$\text{THB (CFU/mL)} = \frac{\text{number of colony}}{\text{dilution x volume plated}}$$

Characterization of bacterial isolates

The morphological and biochemical characteristics of the bacterial isolates were determined using the method described by Cheesbrough (2006). The tests conducted included Gram staining, microscopic examination, and various biochemical tests such as catalase, oxidase, motility, citrate utilization, coagulase, indole production, methyl red–Voges-Proskauer (MR-VP), starch hydrolysis,

and fermentation tests with glucose, lactose, sucrose, and mannitol.

Antibiotic susceptibility testing

For antibiotic susceptibility testing, 2 mL of normal saline was prepared and sterilized by autoclaving at 121°C and 15 psi. After sterilization, the stock isolates were inoculated into the 2 mL of sterile normal saline and incubated at 37°C for 24 hours. Following incubation, the turbidity of the culture was adjusted to match a 0.5 McFarland standard, as outlined in the Clinical and Laboratory Standards Institute (CLSI, 2020) manual.

Antimicrobial susceptibility testing was carried out for all bacterial isolates using the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar (MHA), in accordance with the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS). The bacterial isolates were tested against the following antibiotics with their respective concentrations: Ciprofloxacin (10 µg), Augmentin (30 µg), Tarivid (10 µg), Streptomycin (30 µg), Reflacine (10 µg), Nalidixic Acid (30 µg), Ceporex (10 µg), Septrin (30 µg), Norfloxacin (10 µg), Levofloxacin (20 µg), Ampiclox (20 µg), Chloramphenicol (30 µg), Amoxil (20 µg), Rifampicin (20 µg), Erythromycin (30 µg), and Ampicillin (30 µg).

For testing, biochemically confirmed bacterial isolates grown on nutrient agar were transferred into tubes containing 2 mL of sterile normal saline until they reached the 0.5 McFarland turbidity standard (approximately 1.5×10^8 CFU/mL). A sterile cotton swab was dipped into the bacterial suspension and used to evenly swab the surface of pre-prepared Mueller-Hinton Agar plates. The plates were allowed to dry at room temperature for 5–10 minutes before applying the antibiotic disks. The plates were incubated at 37°C for 18–24 hours. After incubation, the diameters of the zones of inhibition were measured in millimeters and classified as resistant (R) or susceptible (S) based on an interpretive chart.

RESULTS AND DISCUSSION

The results of the microbial counts of the samples are presented in Table 2. The Total Heterotrophic Bacteria (THB) count ranged from 1.7×10^5 to 5.9×10^5 CFU/mL. The Total Coliform Count (TCC) ranged from 1.0×10^3 to 11.5×10^3 CFU/mL. The Total Staphylococcal Count ranged from 1.5×10^4 to 3.2×10^4 CFU/mL.

In the present study, twenty-seven (27) different bacterial isolates were obtained from five different samples. The percentage occurrence of bacterial isolates from all samples is presented in Figure 1. The results show that out of the twenty-seven (27) isolates, *Staphylococcus* spp. accounted for 37%, *Bacillus* sp. for 22%, *Micrococcus* sp. for 4%, *Pseudomonas* sp. for 11%, *Klebsiella* sp. for 7%, and *Escherichia coli* for 19%.

The susceptibility patterns of the bacterial isolates (Gram-positive and Gram-negative) are presented in Tables 3 and 4. The results showed that for Gram-positive organisms, *Bacillus* sp. was mostly resistant to ceftazidime and ciprofloxacin but susceptible to cefuroxime, azithromycin, Amoxil, erythromycin, levofloxacin, and gentamycin. *Staphylococcus* spp. were mostly resistant to cefuroxime and ciprofloxacin but susceptible to rifampicin, levofloxacin, and gentamycin. For Gram-negative organisms, *E. coli* was mostly resistant to cefuroxime while being susceptible to gentamycin and ciprofloxacin. Similarly, *Pseudomonas* sp. was mostly resistant to cefuroxime but susceptible to ofloxacin and ciprofloxacin.

Table 2. Microbial population count of bacterial isolates (CFU/mL).

Sample Code	THB	TCC	TSC
N1	5.9×10^5	11.5×10^3	2.8×10^4
N2	4.7×10^5	1.0×10^3	3.0×10^4
DL1	2.5×10^5	1.0×10^3	1.5×10^4
DL2	3.1×10^5	7.5×10^3	3.2×10^4
DL3	1.7×10^5	3.0×10^3	1.5×10^4

Key: THB - Total Heterotrophic Bacteria, **TCC** - Total Coliform Count, **TSC** - Total *Staphylococcal* Count.

The findings from this study revealed that the total heterotrophic bacterial counts from the different samples showed that for THB and TCC, sample N1 had the highest counts of 5.9×10^5 and 11.5×10^3, respectively. The highest Total Staphylococcal Count was recorded for sample DL2, with a count of 3.2×10^4.

Although recent studies have examined microorganisms associated with dogs, these studies focused more on identifying the organisms (Daodu et al., 2017; Azuonwu et al., 2023). The high microbial counts recorded in this study could be attributed to the overall hygiene levels of the sampled dogs. It may also be related to their nutrition and the time of their last feeding before sample collection, as food residues can influence bacterial counts in the dogs' mouths.

In this study, twenty-seven (27) bacterial isolates

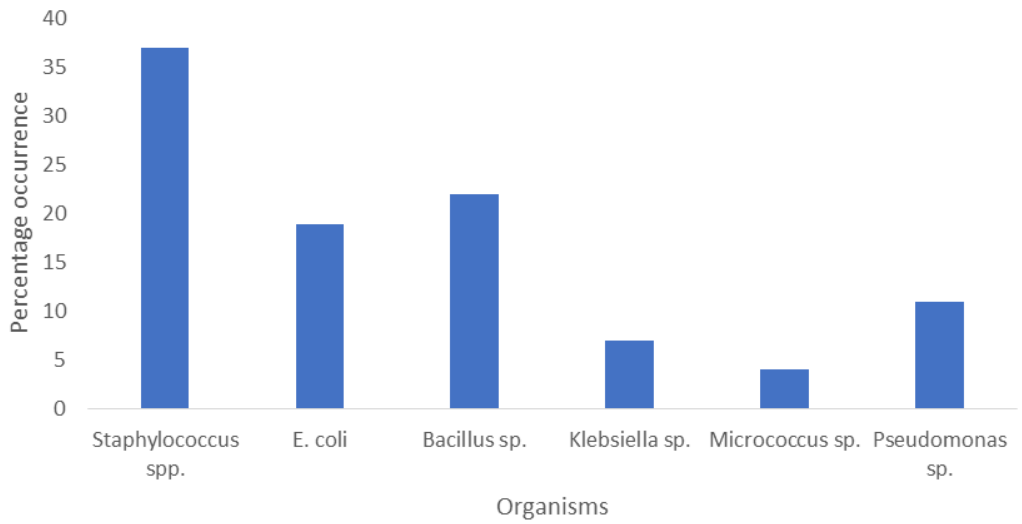

Figure 1. Percentage occurrence of bacterial isolates from all samples.

Table 3. Susceptibility Pattern of *Bacillus* sp., *Staphylococcus* sp. and *Micrococcus* sp. isolated from all samples.

Antibiotics (conc. µg)	***Bacillus sp.*** (6)		***Staphylococcus* sp.** (9)		***Micrococcus* sp.** (1)	
	R	S	R	S	R	S
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Cefuroxime (30µg)	0(0.00)	4(66.7)	4(44.5)	2(22.2)	1(100)	0(0.00)
Rifampicin (20 µg)	2(33.3)	3(50)	1(11.1)	8(88.9)	0(0.00)	1(100)
Ceftazidine (30 µg)	3(50)	1(16.7)	6(66.7)	1(11.1)	0(0.00)	0(0.00)
Streptomycin (30µg)	1(16.7)	2(33.3)	2(22.2)	5(55.6)	0(0.00)	1(100)
Azithromycin (20 µg)	2(33.3)	3(50)	1(11.1)	2(22.2)	0(0.00)	1(100)
Amoxil (10µg)	1(16.7)	4(66.6)	2(22.2)	2(22.2)	0(0.00)	1(100)
Ciprofloxacin (10µg)	2(33.3)	3(50)	4(44.5)	3(33.3)	0(0.00)	1(100)
Erythromycin (30 µg)	1(16.7)	4(66.6)	3(33.4)	3(33.3)	0(0.00)	0(0.00)
Levofloxacin (20 µg)	0(0.00)	6(100)	0(0.00)	9(100)	0(0.00)	1(100)
Gentamycin (10µg)	0(0.00)	6(100)	1(11.1)	7(77.8)	0(0.00)	1(100)

Key: R - Resistant, S - Susceptible.

Table 4. Susceptibility pattern of *E. coli, Klebsiella* sp. and *Pseudomonas* sp. isolated from all Samples.

Antibiotics (conc. µg)	***E. coli*** (5)		***Klebsiella* sp.** (2)		***Pseudomonas* sp.** (3)	
	R	S	R	S	R	S
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Cefuroxime (30µg)	5(100)	0(0.00)	2(100)	0(0.00)	3(100)	0(0.00)
Ofloxacine (10 µg)	0(0.00)	5(100)	0(0.00)	2(100)	0(0.00)	3(100)
Augmentin (30 µg)	1(20)	1(20)	2(100)	0(0.00)	2(66.7)	0(0.00)
Ceftriaxone (30 µg)	1(20)	3(60)	0(0.00)	1(50)	1(33.3)	1(33.3)
Gentamycin (10 µg)	1(20)	4(80)	1(50)	0(0.00)	0(0.00)	1(33.3)
Ciprofloxacin (14µg)	1(20)	4(80)	0(0.00)	2(100)	0(0.00)	3(100)
Streptomycin (30µg)	0(0.00)	3(60)	1(50)	1(50)	1(33.3)	2(66.7)
Peflacine (10 µg)	0(0.00)	3(60)	0(0.00)	2(100)	0(0.00)	2(66.7)
Ceftazidine (30 µg)	3(60)	1(20)	1(50)	1(50)	1(33.3)	1(33.3)
Ceporex (10 µg)	0(0.00)	3(60)	1(50)	1(50)	1(33.3)	2(66.7)

Key: R - Resistant, S - Susceptible.

belonging to six genera were identified, including *Bacillus* sp., *Staphylococcus* spp., *Micrococcus* sp., *Pseudomonas* sp., *Klebsiella* sp., and *Escherichia coli*. Interestingly, the results from this study largely corroborate the findings of Azuonwu et al. (2023), who profiled the antibiogram of bacteria isolated from Eleme

in Rivers State. Their study identified *Bacillus* spp., *Candida albicans*, *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., and *Staphylococcus aureus*, which have been reported as pathogenic and of public health significance (Daodu et al., 2017).

The percentage occurrence of the different isolates in this study was as follows: *Bacillus* sp. (22%), *Staphylococcus* spp. (30%), *Micrococcus* sp. (4%), *Pseudomonas* sp. (11%), *Klebsiella* sp. (7%), and *Escherichia coli* (19%). These findings are similar to but do not completely align with the findings of Azuonwu et al. (2023), who reported prevalence rates of 10%, 21%, 2%, and 20% for *Staphylococcus aureus*, *Escherichia coli*, *Bacillus* spp., and *Klebsiella* spp., respectively. The most frequently occurring organisms in this study were *Staphylococcus* spp. (30%), *Bacillus* sp. (22%), *Escherichia coli* (19%), and *Pseudomonas* sp. (11%).

In a related study by Daodu et al. (2017) on the microbiota of healthy dogs in Ibadan, Nigeria, the percentages of the same organisms with higher occurrence in this study were reported as follows: *Staphylococcus* spp. (14.0%), *Escherichia coli* (18.5%), and *Pseudomonas* sp. (6.8%). However, their study did not report the occurrence of *Bacillus* sp. Discrepancies between studies may be attributed to differences in sample size and type, as well as the conditions under which samples were collected.

Susceptibility testing was performed to determine the antibiogram of the bacterial isolates from the mouths of pet dogs in Port Harcourt using standard antibiotics. The results showed that for Gram-positive organisms, *Bacillus* sp. was mostly resistant to ceftazidime and ciprofloxacin but susceptible to cefuroxime, azithromycin, Amoxil, erythromycin, levofloxacin, and gentamycin. *Staphylococcus* spp. were mostly resistant to cefuroxime and ciprofloxacin but susceptible to rifampicin, levofloxacin, and gentamycin. For Gram-negative organisms, *E. coli* was mostly resistant to cefuroxime while being susceptible to gentamycin and ciprofloxacin. *Pseudomonas* sp. also showed resistance to cefuroxime but was susceptible to ofloxacin and ciprofloxacin.

The results from this study differ from the findings of Daodu et al. (2017), who reported that Gram-positive organisms were susceptible to second-generation cephalosporins like cefuroxime and that susceptibility was highest for ciprofloxacin. In the present study, *Staphylococcus* spp. and *Micrococcus* sp. were resistant to cefuroxime, with susceptibility being highest to levofloxacin and gentamycin. For Gram-negative organisms, *E. coli*, *Klebsiella* sp., and *Pseudomonas* sp. exhibited varying levels of resistance to ofloxacin and ciprofloxacin in Daodu et al. (2017). However, in the present study, these organisms were fully susceptible to these antibiotics. Such differences may be attributed to environmental factors or varying exposure levels to antibiotics in different locations.

A pathogen is classified as multidrug-resistant (MDR) when it is not susceptible to three or more antibiotics (Jan et al., 2004). The antibiogram results from this study show that some of the isolated bacteria can be classified as multidrug-resistant. This finding is consistent with the report by Davis et al. (2014), who documented multidrug-resistant strains of *Staphylococcus* spp. isolated from healthy dogs and cats.

Antibiotic resistance is a growing hazard, as many microorganisms have become resistant to several synthetic antibiotics. The implications of antibiotic resistance for healthcare systems are profound, as resistance limits treatment options. This can result in the failure of drugs to treat microbial infections, which has been associated with the misuse and overuse of antibiotics.

CONCLUSION

In conclusion, potentially pathogenic bacteria are associated with the mouths of pet dogs in the Port Harcourt metropolis. Interestingly, since these dogs serve as pets and sources of emotional support and are in close daily contact with humans, they pose a significant risk of transmitting illnesses caused by the bacteria present in their mouths. Notably, several bacteria of public health importance isolated from the pet dogs in this study exhibited multidrug resistance to antibiotics, further emphasizing their public health significance. This concern is especially relevant in Port Harcourt, where regulations on antibiotic usage are poorly enforced. Such conditions could exacerbate the already critical issue of antibiotic-resistant pathogenic strains, potentially leading to an outbreak in Port Harcourt and its environs. This concern is heightened by the growing trend of pet dog ownership among young people and the broader population in the Port Harcourt metropolis.

Recommendations

Given the presence of potential pathogens in the mouths of pet dogs, it is crucial to prioritize their nutrition and hygiene. Pet dog owners are strongly advised to administer antibiotics to their pets only under the prescription and supervision of a qualified veterinary professional. Additionally, it is essential to closely monitor pet dogs, particularly around children, the elderly, and individuals with weakened or compromised immune systems, to prevent a public health outbreak.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS CONTRIBUTIONS

This work was carried out collaboratively by all authors. Authors OSI and OUA designed the study and drafted the initial manuscript. Author RVK managed the data analysis. All authors read and approved the final manuscript.

DATA AVAILABILITY

Data supporting this study are available upon request from the first or corresponding author.

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Citation: Ogbonna SI, Oparaodu UA, Robinson VK, **2025**. Isolation, characterization and antibiogram of bacteria associated with the mouth of pet dogs in Port Harcourt Metropolis. Microbiol Res Int, 13(1): 1-6.