



ANTIBIOGRAM OF SOME GRAM NEGATIVE BACTERIA ASSOCIATED WITH DOMESTIC DOG FECAL MATTER IN ABAKALIKI, EBONYI STATE

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ABSTRACTS

Although dogs are widely kept as domestic animals and offer many benefits to their owners, it is crucial to remember that pets might have dangerous diseases that can infect humans if prompt precautions are not followed. The aim of this study is to evaluate the antibiogram patterns of some Gram negative bacteria (*Acinetobacter baumannii*, *Escherichia coli*, and *Klebsiella* species) associated with domestic dog faecal matter in Abakaliki Ebonyi state. A total of Ninety two (92) dogs' faecal samples were collected from four areas which include Democracy Estate Residential (23), Onwe Road Residential (23), Mile 50 Residential (23) and Odinukwe Residential (23). Self- structured questionnaire was used to obtain information from the dogs owners. The samples were analyzed using microbiological standard techniques. The Antibiotic susceptibility pattern of the isolates were determined using Kirby-Bauer disk diffusion methods. From the sample analyzed, 92.3 % of the dog owners use them for security. 72.8 % of the dog owners had only 1 dog while 21.7 % had 2- 3 dogs. Dog owners whose dogs were 1 – 6 months were 29.3 % while those with 7 – 10 months were 21.7 %. Owners of German shepherd was 25 % while bull dogs were 20.7 %, Labrador retriever dogs were 32.6 % and local dogs were 21.7 %. About 50 % vaccinate their dogs once yearly while 37 % and 13 % vaccinate their dogs twice a year. The bacterial isolates were Gram negative bacteria viz: *Acinetobacter baumannii*, *Escherichia coli*, and *Klebsiella* specie with frequency of 12.2 %, 8.2 % and 79.6 %, respectively. 33.3 % *Klebsiella* species were isolated from Mile 50 and Democracy Estate, 16.7 % were isolated from Odinukwe residential area and Onwe Road. However, the *Acinetobacter baumannii*, none were isolated from Onwe Road, 50 % were isolated from democracy estate while 25 % each were isolated from Odinukwe and Mile 50. *Escherichia coli* was the highest bacteria organism isolated representing (39/49) (79.6 %) of all bacteria isolated. Distribution of *Escherichia coli* in different locations showed that 33.3% were from Mile 50, 25.6 % were from Odinukwe and Democracy Estate while 15.3 % were isolated from domestic dog fecal samples from Onwe Road. Most antibiotic such as nitrofurantoin (98.9 %) amoxicillin (96.1 %) and ceftrazone (88.3 %) were resistant to the bacteria isolate from Odinukwe residential, Mile 50 residential, Democracy estate residential and Onwe Road Residential. It is important to implement strict sanitation measures to reduce the environmental impact of bacteria from dog fecal matters. It is therefore recommended that dog's owners should properly vaccinate their dogs accordingly.

Key words; Antibiogram, Domestic dog fecal matter, Antibiotic resistance, Microbial isolation

INTRODUCTION

Dogs are domesticated carnivorous mammals that typically has a long snout, and acute sense of smell, non-retractable claws and barking howling or whining voice (Thalmann *et al.*, 2018). The types of dogs include German shepherd, bull dogs, Labrador retriever and local dogs. The risk of zoonotic infections has increased due to the rise in the number of stray domestic and semi-domestic dogs in metropolitan areas around the world. Most often, domestic dogs are maintained as either pets or for security purposes. According to the World Health Organization (2018), over 5 million individuals are bitten by dogs worldwide each year (WHO, 2018). This clear method is used by dogs to spread numerous parasitic and zoonotic illnesses (Obioma *et al.*, 2020). Consequently, a huge majority of diseases that affect humans today are zoonotic infections that are transmitted from animals.

Even though having a domestic animal has numerous advantages, as seen in communities all over the world, it is important to note that sometimes hazardous bacteria are carried by pets, which can make people sick if preventive measures are not taken in a timely manner. Even so, diseases that humans contract from animals are known as zoonotic diseases, and it can be challenging to identify which animals may be carriers (Center for Disease Control, 2014). This is because the majority of animals that carry these pathogens frequently appear healthy and normal, making them obvious asymptomatic carriers.

Dogs are vulnerable contract zoonotic diseases from a variety of pathogens, including bacteria, fungus, viruses, and parasites. Ringworm, salmonellosis, leptospirosis, lyme disease, campylobacter infection, giardia infection, cryptosporidium infection, roundworms, hookworms, tapeworms, scabies, harvest mites, and rabies are the most prevalent zoonotic diseases that affect dogs. Zoonosis has been identified as one of the causes of death of gastrointestinal tract diseases, with diarrhea being one of the most prevalent clinical presentations and having the ability to cause severe dehydration (Marks *et al.*, 2011; Grellet *et al.*, 2012).

However, domestic dogs, and pets in general, provide comfort and company, and one cannot but love them. While there are many ways that having pets can improve our physical and mental health, they have also likely contributed to a number of ailments that affect people worldwide (Koivusilta and Ojanlatva, 2016). Since dog feces are a public health hazard, various microbes were recovered from dog feces in an Italian investigation, including *Salmonella*, *Yersinia* or *Campylobacter* species, *Giardia* cysts, *Enterococcus* species, *E. faecium*, *E. gallinarum*, *E. casseliflavus*, and *E. faecalis* (Cinquelpalmi *et al.*, 2012).

However, as indicated by several research both inside and outside the territory of the present study, the antibiogram pattern of potentially harmful bacteria linked to domestic dogs exhibits a high degree of variation in the various geographic landscapes. The resistance and susceptibility rate and pattern was maintained in several investigations, nevertheless. However, it is firmly believed that since the early 20s, there has been an increased indication that pets and pet feces may likely be a reservoir for dangerous antibiotic-resistant bacteria strains, posing a new threat to public health intervention programs and strategies across the global communities. Various antibiotics, such as clindamycin, vancomycin, tetracycline, erythromycin, and ampicillin, have been reported to contain some strains that are resistant to them (Cinquelpalmi *et al.*, 2012). Given the general medical implications of such outcomes in our developing communities with weak, fragile health facilities to handle such complicated, and non-cost-effective treatment options among the poor citizens, these should be of concern to microbiologists, clinicians, and other numerous stakeholders in the medical profession.

The MRSA isolated from companion animals is similar to MRSA strains obtained in a hospital setting, indicating that these strains may be transmitted from animals to humans or vice versa. The description of the isolation of dogs-produced extended-spectrum beta-lactamases by *Escherichia coli* is further cause for concern (Obioma *et al.*, 2020).

Due to the abuse of antibiotics in companion animals, dogs thus offer a potential source of bacteria that are resistant to antibiotics. A variety of mammals' large intestines are colonized by anaerobic, mobile, gram-negative bacteria called *Salmonella* species, particularly in the canine's mesenteric lymph nodes and the distal portion of the colon. Additionally, humans are susceptible to a number of infectious disorders including gastroenteritis, enteric fever, bacteremia, and osteomyelitis through gastrointestinal tract infection (faecal transmission). The majority of infected dogs and humans are asymptomatic and may shed the pathogen through feces for a period of 6 weeks, during which time they may spread the pathogen to other animals or people nearby. Gastrointestinal illnesses are the most common clinical presentations of *Salmonella* in humans and dogs, but they are not the only ones.

Salmonella species are more common in poor nations than they are in industrialized nations (Leonard, 2014). For patients with infections caused by *Salmonella* species, an antibiogram laboratory assay should be taken into consideration. The infection may be treated with antibiotics from different families, such as fluoroquinolones,

beta-lactams, and macrolides, but performing the analysis first is still essential for the specificity and sensitivity of the final result (Leonard *et al.*, 2011).

1.2. Statement of Problem

Concerns regarding the zoonotic spread of AMR have led to reports in a number of investigations that AMR has become an increasing problem in companion animal (dog) isolates over time (Wong *et al.*, 2015). Dog feces have also been shown to contain Methicillin-Resistant *Staphylococcus aureus* (MRSA), which has been isolated from both sick and colonized pet animals (Obioma *et al.*, 2020).

Potentially pathogenic microorganisms are linked to risk factors and public health implications in domestic dogs. Breed, gender, immunization history, age, season, breeder-origin, and/or kennel stay are putative risk factors for canine acute infectious diarrhoea (Bagshaw *et al.*, 2014). These risk factors for infection with certain enteropathogens have, however, primarily been documented in dogs living in shelters or breeding facilities. For privately owned dogs kept in various living situations, the significance of some of these risk factors may vary.

The lack of information on the aforementioned topic area, however, sparked the motivation and enthusiasm for the investigation. Additionally, the researchers were attracted to investigate this line of inquiry due to the conflicting outcomes from the limited number of studies that had previously been conducted, particularly given the rising demand for dogs as pets and for security purpose.

1.3. Significant of the Study

The study will add to the existing knowledge on the degree of resistant Gram negative bacteria from dog faecal contamination, and help in putting measure against the spread of pathogenic bacteria. Also, this study will help policy makers on enacting laws towards proper managements of domestic dog. This research will educate dog owners on the proper disposal of dog faecal waste or faecal matter in Nigeria. More so, it will point towards informing the dog owners on proper vaccinations of their dogs

1.4. Aim of the Study

The aim of this study is to evaluate the antibiogram patterns of some Gram negative bacteria (*Acinetobacter baumannii*, *Escherichia coli*, and *Klebsiella* species) associated with domestic dog faecal matter in Abakaliki Ebonyi state

1.5. Objective of the Study

The objective of this study were to:

- I. Isolate and identify some Gram negative Bacteria (*Acinetobacter baumannii*, *Escherichia coli*, and *Klebsiella* species) that are associated with domestic dog fecal matter in Abakaliki Ebonyi State.
- II. Determine the distribution of Gram negative (*Acinetobacter baumannii*, *Escherichia coli*, and *Klebsiella* species) bacteria that are associated with Domestic Dog fecal matter.
- III. Determine the susceptibility patterns of Gram negative (*Acinetobacter baumannii*, *Escherichia coli*, and *Klebsiella* species) bacteria associated with domestic dog fecal matter.

MATERIALS AND METHODS

Study Area

The study area was in Abakaliki (Democracy Estate residential, Onwe road residential, mile 50 residential and Odinukwe residential) Ebonyi State, Nigeria. Abakaliki has a land mass of 51 km² and a population of 583,303. The major occupation of the populace is farming and trading. The area lies on latitude 6°22'26"N and longitude 8°6'6" E of the Greenwich meridian. The rainfall pattern is moderate with average atmospheric temperature of 32-35°C.

3.2. METHODS

3.2.1. Sample collection

A total of Ninety two (92) dogs' faecal samples were collected from four areas which include Democracy Estate Residential (23), Onwe Road Residential (23), Mile 50 Residential (23) and Odinukwe Residential (23). The samples were collected using applicator stick into a sterile container. It was immediately transported to Microbiology Laboratory Complex for bacteriological analysis.

3.2.2. Preparation of Nutrient broth

Nutrient broth was prepared by dissolving 3.9 g of nutrient broth powder in 300 ml of distilled water according to the manufacturer's instructions. After that, 5 ml of prepared nutrient broth was added aseptically to the capped test tubes and sterilized by autoclaving at 121 °C for 15 mins at 15 psi (Cheesbrough, 2010).

3.2.3. Preparation of Nutrient agar

Nutrient agar was prepared by dissolving 5.6 g of nutrient broth powder in 200ml of distilled water according to the manufacturer's instructions. After that 5ml of prepared nutrient broth was added aseptically to petri di and sterilized by autoclaving at 121 for 15mins at 15psi (Cheesbrough, 2010). Sub culturing was carried out to isolate a pure colony.

3.2.4. MacConkey Agar

A filter paper was placed on a weighing balance, 17,6 grams of the MacConkey agar powder was weighed, the weighed agar powder was poured into a beaker, 1000 ml of distilled water measured using a measuring cylinder, the measured water was poured into a beaker and stirred using the spatula to dissolve the agar powder uniformly, the opening of the beaker was sealed using cotton wool and aluminum foil and was placed inside an autoclave to sterilize, the medium was sterilized by autoclaving at a temperature of 121 °C for 15 minutes at a pressure of 15 PSI, the pressure and temperature of the autoclave was allowed to cool and the lid was opened to remove the sterilized medium, the media was allowed to cool down and was poured aseptically into sterile Petri dishes and allowed to air dry, the plates was placed inside the incubator to dry the solidified media. The sterilized plates were examined for sterility by incubating at 30 to 35°C for 24 h or longer (Cheesbrough, 2006).

3.3. Bacteriological Analysis

3.3.1. Isolation

The samples were cultured in the various media and incubated for 24 hours at 38°C.

3.3.2. Gram Reaction

A dry smear was made on a slide and heat fix by passing it over flame, the fixed smear will be covered with crystal violet stain for 30 to 60 seconds, the stain was rapidly washed off with clean water, all the water on the slide will be tipped off, the smear was covered with Lugol's iodine for 30 to 60 seconds, the iodine was washed off with clean water and was decolorize rapidly (few seconds) with acetone–alcohol, it was immediately washed off with clean water, the smear was covered with neutral red stain for 2 minutes, the stain was washed off with clean water, the back of the slide was wiped clean and placed on a draining rack for the smear to air-dry, then the smear was examined microscopically, first with the 40X objective lens to check the staining and to see the

distribution of material, and then with the oil immersion objective to report the bacteria and cells (Cheesbrough, 2006).

3.4. Biochemical Tests.

3.4.1. Catalase Test

Two to three (2 to 3 ml) of the hydrogen peroxide solution will be poured into test tubes, using a sterile wooden stick or a glass rod (notanichrome wire loop), several colonies of the test organism was removed and immerse in the hydrogen peroxide solution and then immediate bubbling was checked for. Active bubbling showed positive catalase test, no bubbles showed negative catalase test (Cheesbrough, 2006).

3.4.2. Indole Test

Detecting indole using tryptone water (Kovac's reagent)

The test organism were inoculated in a bijou bottle containing 3 ml of sterile tryptone water, the mixture were incubated at 35–37°C for up to 48 h, indole was tested by adding 0.5 ml of Kovac's reagent and shaken gently, a red colour was examined in the surface layer within 10 minutes. Red surface layer indicated positive indole test, no red surface layer indicated negative indole test (Cheesbrough, 2006).

3.4.3. Citrate Utilization Test

Citrate method using Simmon's citrate agar

Slopes of the medium was prepared in bijou bottles as recommended by the manufacturer (stored at 2–8°C), the slope was first streaked using a sterile straight wire with a suspension of the test organism and then stab the butt, the stabbed slop was incubated at 35°C for 48 h. A bright blue colour was examined in the medium. Bright blue showed positive citrate test, no change in colour showed negative citrate test of medium (Cheesbrough, 2006).

3.4.4. Oxidase Test

A filter paper was soaked with the substrate tetramethyl-p-phenylenediamine dihydrochloride, the paper was moistened with sterile distilled water, and the colony to be tested was picked with wooden or platinum loop and smeared in the filter paper and then observed inoculated area of paper for a color change to deep blue or purple within 10-30 seconds. Oxidase positive organisms gave blue color within 5-10 seconds, and in oxidase negative organisms, color does not change (Cheesbrough, 2006).

3.4.5. Methyl Red Test

Single colony from the pure culture of the test organism was inoculated in 5 ml of sterile MR-VP broth and incubation at 37 °C for 48 h. After this, 3 – 5 drops of methyl red solution was added and observed for colour formation. Development of red or yellow colour indicated positive or negative result, respectively (Cheesbrough, 2006).

3.4.6. Motility Test

A premixed commercially available motility medium were prepared according to manufacturers' instruction. The medium were brought to boil, dispensed into 10ml test tube and sterilized by autoclaving at 121 °C for 15 minutes. Sterilized medium was cooled in an upright position on racks. Using a sterile inoculating needle, the motility medium were be inoculated with isolated colony of the test organism by making a fine stab to a depth of about 1-2 cm short of the bottom of the tube. The tubes were then be incubated at 37 °C for 24 h. The line of inoculation were not sharply defined and the rest of the medium were somewhat cloudy if the organism is motile. The growth of non-motile organisms were restricted to the line of inoculation which became sharply defined and the rest of the medium remained clear (World Health Organization/Centers for Disease Control and Prevention/ United States Agency for International Development, WHO/CDC and P/USAID, 2003).

3.5. Preparation of Mueller-Hinton agar

This was prepared by measuring 38 g of the powdered agar into 1 liter of distilled water. It mix thoroughly and heated with frequent agitation and boil for 1 minute to completely dissolve the components. Then it was autoclaved at 121 °C for 15 minutes. This was allowed to cool at 45 °C and the cooled Mueller Hinton agar were aseptically poured into sterile Petri dishes on a level, horizontal surface to give uniform depth. It was left to solidify at room temperature.

3.5.1. Antibiotic Susceptibility Testing

The susceptibility of test organism to antibiotics were determined by modified Kirby-Bauer disk diffusion methods on Muller Hinton agar. Their susceptibility to various antibiotics were tested. Using a freshly prepared Muller-Hinton agar, a suspension of organism were made equivalent to 0.5 McFarland's turbidity standard. A sterile swab stick were dipped into the suspension, pressed and rotated firmly against the side of the tube to remove excess liquid and then swabbed evenly across the surface of the plate. After which the disc were

placed using forceps at about 15 mm from the edges of the plate and no closer than 25 mm from disc to disc after inoculation of organism. After overnight incubation at 37 °C for 24 hrs, the diameters of zones of inhibition produced were determined. Zones of inhibition were measured with meter rule in (mm) and were interpreted as either susceptible, resistance or no inhibition, using the NCCLS method.

3.6. Statistical Analysis

Data was collected using Microsoft Excel and transferred into Statistical Package for Social Science (SPSS) for statistical analysis. Critical consideration was given to statistical analysis SPSS version 21 to analyses the data for frequency, percentage. Results were presented in tables only.

RESULTS

4.1. Information of dog and dogs` owner in Abakaliki metropolis

The result in Table 1 showed that all the dog owners had different reasons of keeping them, majority (92.3 %) of the dog owners said that the reason of having dog was for security while 7.6 % said it serve as domestic pets. Many (72.8 %) of the dog owners had only 1 dog while 21.7 % had 2- 3 dogs and 5.4 % participants said they had 2 -3 dogs. Dog owners whose dogs were 1 – 6 months was 29.3 % while those with 7 – 10 months gave 21.7 % and those with 1 year was 35.9 % as well as 2 years being 13 %.

Owners of German shepherd was 25 % while bull dogs was 20.7 % and Labrador retriever dogs was 32.6 % however, local dogs was 21.7 %.

According to the dog owners, the types of food given to their dogs were fresh food, cooked food, canned food and foods from waste bin with the following percentage 8.7, 56.5, 9.8 and 25, respectively. Many (85.9 %) of the respondent said that their dog do not go out of their compound while 14.1 % said that their dog usually go out (Table 1).

The dog owners that vaccinate their dogs were 96.7 % while 3.3 % were not vaccinated. Fifty percent of dog's owners said they vaccinate their dogs once yearly while 37 % and 13 % vaccinate their dogs twice and none respectively. Many (97.9 %) of the dog owners said their dogs had develop sickness while 3.1 % opposed. Ninety six point three (96.3 %) of the said dogs had not caused any sickness within the household while 3.3 opposed.

Many (84.8 %) of the participants said they have a veterinary that check-up their dogs while 15.2 % do not (Table 1).

4.2. Prevalence of *Escherichia coli* *Acinetobacter baumannii* and *Klebsiella* species isolated from dogs types from various locations in Abakaliki.

The table below represent prevalence of *Escherichia coli* *Acinetobacter baumannii* and *Klebsiella* species isolated from dog's types from various locations in Abakaliki

E. coli and *Klebsiella* species isolated from local dogs in Odinukwe residential area had the highest percentage 4(20.0 %) and 1 (5.0 %) compared to other dog types.

In mile 50 Residential area, Local dog had the highest percentage of *E. coli* 5 (25.0 %) followed by Labrador retriever 4 (13.3 %), Bull dog 2 (10.5 %) and German shepherd 2(8.7 %). however, the percentage of *Acinetobacter baumannii* and *Klebsiella* species isolated from local dog were 1(5.0 %) respectively.

E. coli was found to be the most isolated bacterial from local dog's types from Democracy Estate residential with 5(25.0 %) followed by *Acinetobacter baumannii* and *Klebsiella* species.

Local dogs from Onwe Road residential Area had *E. coli* as the highest isolate bacteria with 2 (10.0 %) followed by Labrador retriever 2 (6.7 %), Bull dog (5.3 %) and German shepherd 1 (4.3 %) however, *Klebsiella* species 1 (5.0 %) was also isolated from local dogs.



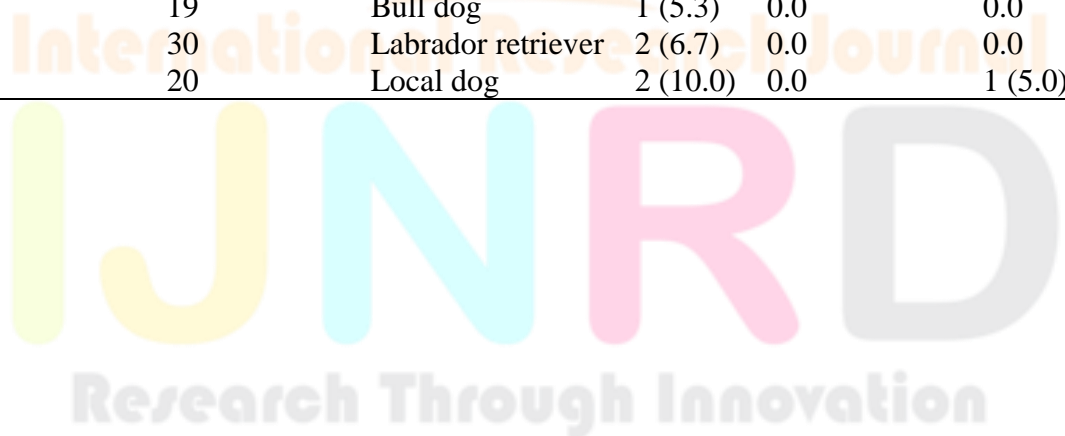
Table 1. Information of dog and dogs` owner in Abakaliki Metropolis

Indications	Variable	Frequency (n= 92)	Percentages (%)
Purpose of dogs keeping?	Pet	7	7.6
	Security	85	92.3
Number of dogs?	1	67	72.8
	2-3	20	21.7
	3 and above	5	5.4
Dog Age (months/years)	1 – 6 months	27	29.3
	7 – 10 months	20	21.7
	1 year	33	35.9
	2 years	12	13.0
Dog Types (species)	German shepherd	23	25.0
	Bull dog	19	20.7
	Labrador retriever	30	32.6
	Local dog	20	21.7
Nutrition status of the dogs	Fresh food	8	8.7
	Cook food	52	56.5
	canned food	9	9.8
	Feeds from waste bin	23	25.0
Do your dog(s) go out of the compound?	Yes	13	14.1
	No	79	85.9
Vaccination Status	Yes	89	96.7
	No	3	3.3
Degree of vaccination	Once	46	50.0
	Twice	34	37.0
	None	12	13.0
Zoonotic disease (s) experienced	Yes	90	97.9
	No	2	3.1
	No	89	96.3
Veterinary doctor involvement	Yes	78	84.8
	No	14	15.2
Degree of dogs check up by veterinary doctors	Regularly	34	37.0
	Once in 6 months	12	13.0
	Once a year	46	50.0

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Table 2. Prevalence of *Escherichia coli* *Acinetobacter baumannii* and *Klebsiella* species**isolated from dogs types from various locations in Abakaliki**

Locations	No of dog fecal samples analyzed	Dog species	<i>E. coli</i> (%)	<i>Acinetobacter baumannii</i> (%)	<i>Klebsiella</i> species (%)
Odinukwe Residential Area	23	German shepherd	2 (8.7)	0.0	0.0
	19	Bull dog	2 (10.5)	0.0	0.0
	30	Labrador retriever	2 (6.7)	1 (3.3)	0.0
	20	Local dog	4 (20.0)	0.0	1 (5.0)
Mile 50 Residential Axis	23	German shepherd	2(8.7)	0.0	0.0
	19	Bull dog	2 (10.5)	0.0	0.0
	30	Labrador retriever	4 (13.3)	0.0	1 (3.3)
	20	Local dog	5 (25.0)	1 (5.0)	1 (5.0)
Democracy Estate Residential	23	German shepherd	1 (4.3)	0.0	0.0
	19	Bull dog	2 (10.5)	1(5.3)	0.0
	30	Labrador retriever	2 (6.7)	1 (3.3)	0.0
	20	Local dog	5 (25.0)	0.0	2 (10.0)
Onwe Road Residential Area	23	German shepherd	1 (4.3)	0.0	0.0
	19	Bull dog	1 (5.3)	0.0	0.0
	30	Labrador retriever	2 (6.7)	0.0	0.0
	20	Local dog	2 (10.0)	0.0	1 (5.0)



4.3. Morphological and Biochemical Characterization of the Bacterial isolates from Domestic Dogs fecal Matter in Abakaliki

Morphological and biochemical characterization of bacterial isolated from domestic dogs fecal matter sampled from the various locations within Abakaliki revealed a total of three (3) bacterial isolates which were all Gram negative bacteria (*Acinetobacter baumannii*, *Escherichia coli*, and *Klebsiella* specie).



Table 3. Biochemical characteristics of bacterial isolates from Domestic Dog fecal Matter in Abakaliki.

Colonies feature	Morphology	Gram Stain	Indole	Oxidase	Catalase	Citrate	Motility	Methyl Red	Possible Organisms
Opaque, and circular colonies on MA agar and circular, and gray color in NA	Rod	-	+	-	+	+	-	-	<i>Acinetobacter baumannii</i>
Pink to dark pink, dry and donut shaped on MA and large, thick, greyish white, moist, smooth, opaque on NA	Rod	-	+	-	+	-	-	-	<i>Escherichia coli</i>
creamy white, circular, raised, fluidal on NA large, mucoid, and pink to red in color on MA	Rod	-	+	-	+	+	-	-	<i>Klebsiella</i> species

Key: + = positive, - = negative.

4.4. Frequency of occurrence of the bacteria isolate in the samples

Out of ninety two (92) samples collected, Gram negative bacteria such as *Klebsiella* species, *Acinetobacter baumannii* and *Escherichia coli* were isolated in forty nine samples with a frequency of 12.2 %, 8.2 % and 79.6 % respectively (Table 4).

4.5. Frequency of bacterial organisms isolated based on different Locations

Table 4 showed the frequency of bacterial isolated from dog fecal matter isolated from different location, while 33.3 % *Klebsiella* species was isolated from Mile 50 and Democracy Estate, 16.7 % was isolated from Odinukwe residential area and Onwe Road. However, the *Acinetobacter baumannii*, none was isolated from Onwe Road, 50 % was isolated from democracy estate while 25 % each were isolated from Odinukwe and Mile 50.

Escherichia coli was the highest bacteria organism isolated representing (39/49) (79.6 %) of all bacteria isolated. Distribution of *Escherichia coli* in different locations showed that 33.3% was from Mile 50, axis 25.6 % was from Odinukwe and Democracy Estate while 15.3 was isolated from domestic dog fecal samples from Onwe Road (Table 4)



Table 4. Occurrence of the bacteria isolated from dog fecal matter in Abakaliki**Metropolis**

Bacteria organisms	Frequency	Percentage (%)
<i>Klebsiella</i> species	6	12.2
<i>Acinetobacter baumannii</i>	4	8.2
<i>Escherichia coli</i>	39	79.6
Total	49	100



Table 5. Frequency of bacterial isolates based on different Locations in Abakaliki metropolis

Locations	<i>Klebsiella</i> species	<i>Acinetobacter</i>	<i>Escherichia coli</i>
	(%)	<i>baumannii</i> (%)	(%)
Odinukwe Residential Area	1 (16.7)	1 (25.0)	10 (25.6)
Mile 50 Residential Axis	2(33.3)	1 (25.0)	13 (33.3)
Democracy Estate Residential	2(33.3)	2 (50.0)	10 (25.6)
Onwe Road Residential Area	1 (16.7)	-	6 (15.3)
Total	6	4	39

4.6. Antibiotics Resistance and susceptibility pattern of *Escherichia coli* *Acinetobacter baumannii* and *Klebsiella* species isolated based on locations from Domestic Dog fecal Matter in Abakaliki

Results of the antibiotic susceptibility pattern based on various locations are represented in table 6. In Odinukwe residential, *Escherichia coli* was 100 % susceptible to ciprofloxacin, perfloxacin, ofloxacin and 80 % susceptible to gentamicin and streptomycin. However the isolate was resistant to chloramphenicol (50 %) and meropenem (60.0 %). *Escherichia coli* was 10 % resistant to nitrofurantoin. Also, in Mile 50 residential, *Escherichia coli* susceptibility pattern was 100 % to ciprofloxacin, perfloxacin, ofloxacin and ceftrazone were 84.6 % respectively, chloramphenicol (69.2 %), gentamicin (61.5 %), meropenem and streptomycin (53.8 %). Susceptibility pattern of *Escherichia coli* isolated from Democracy estate residential was ofloxacin (100 %), perfloxacin (90 %), gentamicin, ciprofloxacin and chloramphenicol showed 80 %, while meropenem and ceftrazone gave

60 % and streptomycin (50. %). Susceptibility pattern of *Escherichia coli* isolated from Onwe Road residential were gentamicin, ciprofloxacin ofloxacin and perfloxacin (100 %), streptomycin (83.3 %) and chloramphenicol and meropenem (66.7 %) and ceftrazone (50.0 %) (Table 6).

Acinetobacter baumannii isolated from Odinukwe residential Area showed 100 % resistant to ciprofloxacin, ofloxacin and perfloxacin. The isolate was 100 % susceptible to gentamicin. The antibiogram pattern of *Acinetobacter baumannii* isolated from dogs fecal matter in Mile 50 residential showed 100 % resistant to nitrofurantoin, gentamicin, perfloxacin and Amoxicilin and 100 % susceptible to ciprofloxacin, chloramphenicol and meropenem. While the isolate from Democracy Estate Residential Area was 100 % resistant to gentamicin, meropenem, perfloxacin and ceftrazone as well as 50 % resistant to streptomycin and ofloxacin while susceptibility was observed with ciprofloxacin (Table 6).

Klebsiella species isolated from dog fecal matter in Odinukwe residential show 100 % susceptibility in gentamicin, ciprofloxacin, chloramphenicol, ofloxacin, perfloxacin, ceftrazone and streptomycin and 100 % resistant to meropenem. In Mile 50 residential, the isolate showed 100 % resistant to chloramphenicol, perfloxacin and streptomycin and 50 % resistant to gentamicin, ciprofloxacin, meropenem and ceftrazone. Susceptibility was observed with ofloxacin (100 %). Isolate from Democracy estate residential was 100 % susceptible to nitrofurantoin, gentamicin, ciprofloxacin and perfloxacin. 100 % resistant was observed with chloramphenicol and ceftrazone (100 %). Isolate from Onwe road residential showed 100 % resistant to gentamicin, ciprofloxacin and ceftrazone as well as 100 % susceptibility to chloramphenicol, ofloxacin and perfloxacin (Table 6).

Table 6. Antibiotics Resistance and susceptibility pattern of *Escherichia coli* *Acinetobacter baumannii* and *Klebsiella* species isolated based on locations from Domestic Dog fecal Matter in Abakaliki

Location	Bacteria		N	GEN	CIP	CHL	OF	ME	PE	CEF	STR	AM
Odinukwe	<i>Escherichia coli</i>	R	1(10.0)	2(20.0)	0.0	5(50.0)	0.0	6(60.0)	0.0	4(40.0)	2(20.0)	-
		S	-	8(80.0)	10(100)	5(50.0)	10(100)	4(40.0)	10(100)	6(60.0)	8(80.0)	-
	<i>Acinetobacter baumannii</i>	R	-	0.0	100	-	100	-	100	-	-	-
		S	-	100	0.0	-	0.0	-	0.0	-	-	-
	<i>Klebsiella species</i>	R	-	0.0	0.0	0.0	0.0	100	0.0	0.0	0.0	-
		S	-	100	100	100	100	0.0	100	100	100	-
Mile 50 Residential Area	<i>Escherichia coli</i>	R	1(7.69)	2(15.4)	0.0	4(30.8)	2(15.4)	6(46.2)	2(15.4)	2(15.4)	6(46.2)	3 (23.1)
		S	1(7.69)	8(61.5)	13(100)	9(69.2)	11(84.6)	7(53.8)	11(84.6)	11(84.6)	7(53.8)	-
	<i>Acinetobacter baumannii</i>	R	100	100	0.0	0.0	-	0.0	100	-	-	100
		S	0.0	0.0	100	100	-	100	0.0	-	-	0.0
	<i>Klebsiella species</i>	R	-	1(50.0)	1(50.0)	2(100)	0.0	1(50.0)	2(100)	1(50.0)	2(100)	1(50.0)
		S	-	1(50.0)	1(50.0)	0.0	2(100)	1(50.0)	0.0	1(50.0)	0.0	1(50.0)
Democracy Estate Residential Area	<i>Escherichia coli</i>	R	3 (23.1)	2(20.0)	2(20.0)	2(20.0)	0.0	4 (40.0)	1(10.0)	4 (40.0)	5(50.0)	-
		S	-	8(100)	8(100)	8(100)	100	6(60.0)	9(90.0)	6(60.0)	5(50.0)	-
	<i>Acinetobacter baumannii</i>	R	-	2(100)	0.0	-	1(50.0)	2(100)	2(100)	2(100)	1(50.0)	-
		S	-	0.0	2(100)	-	1(50.0)	0.0	0.0	0.0	1(50.0)	-
	<i>Klebsiella species</i>	R	0.0	0.0	0.0	2(100)	1(50.0)	1(50.0)	0.0	2(100)	1(50.0)	-
		S	2(100)	2(100)	2(100)	0.0	1(50.0)	1(50.0)	2(100)	0.0	1(50.0)	-
Onwe Road Residential Area	<i>Escherichia coli</i>	R	-	0.0	0.0	2(33.3)	0.0	2(33.3)	0.0	3(50.0)	1(16.7)	-
		S	-	6(100)	6(100)	4(66.7)	6(100)	4(66.7)	6(100)	3(50.0)	5(83.3)	-
	<i>Klebsiella species</i>	R	-	100	100	0.0	0.0	-	0.0	100	-	-
		S	-	0.0	0.0	100	100	-	100	0.0	-	-

KEY: N= Nitrofurantoin (300 µg), **GEN** = Gentamicin (10 µg), **CIP** = Ciprofloxacin (10 µg), **CHL** = Chloramphenicol (10 µg), **OF** = Ofloxacin (10 µg), **ME** = Meropenem (10 µg), **PE** = perfloxacin (10µg), **CEF** = Ceftrazone (30µg), **STR** = Streptomycin (10µg) and **AM** = Amoxicilin (30µg)

Research Through Innovation

DISCUSSION OF RESULTS

5.1. Discussion

In urban or rural areas, dog feces may be a significant source of potentially dangerous bacteria that could contaminate both dog owners and the overall community ecosystem, making the area unfit for human settlement. However, Koivusilta and Ojanlatva (2016), found that domestic dogs, including pet dogs, in our communities transmit illnesses with many causal agents.

In this study, almost all respondents (97.9 %) claim to have experienced zoonotic diseases, and a significant number (84.8 %) consult veterinary doctors. Zoonotic diseases are prevalent, highlighting potential risks to both dogs and owners. Regular veterinary care is essential for preventing and managing these diseases. The high percentage of veterinary consultations indicates a responsible approach to pet care (Ateudjieu *et al.*, 2023; Bayko *et al.*, 2023). A majority (85.9 %) of dogs do not go out of the compound, and almost all (96.7 %) are vaccinated, this suggest that limited outdoor exposure may affect dogs' physical and mental stimulation, while high vaccination rates suggest a commitment to their health (Okeme *et al.*, 2020). The implication is that dog owners might prioritize the health of their dogs over allowing them of extensive outdoor activities (Westgarth *et al.*, 2019).

Based on this study, the bacterial organisms identified from dogs fecal matter were *Acinetobacter baumannii*, *Escherichia coli* and *Klebsiella* species. Presence of potentially pathogenic bacteria in dog fecal matter indicates a potential risk to public health. Similar biochemical characteristics among the identified bacteria suggest a commonality in their metabolic processes. The presence of *Escherichia coli* indicates fecal contamination and a potential source of gastrointestinal infections. This in line with a study conducted to detect antibiotic-resistant canine origin *Escherichia coli* and the synergistic effect of magnolol in reducing the resistance of multidrug-resistant (MDR) *Escherichia coli* by Tong *et al.* (2023). He reported that a total of 101 *E. coli* strains were isolated from 158 dog fecal samples collected from animal hospitals and that MIC determinations showed that 75.25 % (76/101) of the *E. coli* strains were MDR. Hata *et al.*

(2022) conducted on surveillance of antimicrobial-resistant *Escherichia coli* in sheltered dogs in the Kanto Region of Japan and reported that *E. coli* was detected in the fecal samples of all 61 and 77 shelter dogs tested in Chiba and Kanagawa, respectively. Boonmasawai *et al.* (2018) reported that the prevalence of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae among the bacterial strains isolated from the dogs was 70.1%. Moreover, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Staphylococcus aureus* were isolated from the rectal swabs.

In this study, *Escherichia coli* was the most frequently occurring bacteria, with a percentage of 79.6 %, while *Klebsiella* species and *Acinetobacter baumannii* had percentages of 12.2 % and 8.2 %, respectively. A similar study was conducted by Bourély *et al.* (2019). High frequency of *Escherichia coli* indicates a significant presence of fecal contamination. *Klebsiella* species and *Acinetobacter baumannii*, though lower, still pose potential health risks. *Escherichia coli*'s dominance suggests a substantial contribution of dog fecal matter to environmental contamination.

Mile 50 residential area has the highest frequency of *Klebsiella* species (33.3 %) and *Escherichia coli* (33.3 %). *Acinetobacter baumannii* has the highest frequency (50.0 %) in Democracy Estate Residential Area. Odinukwe Residential Area has relatively lower frequencies for all the three bacteria. Varied frequencies across locations suggest potential differences in environmental factors influencing bacterial presence. Higher frequencies of *Escherichia coli*, *Acinetobacter baumannii* and *Klebsiella* species isolated from dogs' fecal matter in specific areas may indicate varying levels of hygiene or environmental contamination. Local factors (environmental conditions) may influence the high frequency of this pathogens at various locations. Among the Gram-negative bacteria from cases of canine pyoderma in Grenada (West Indies), the most common species isolated was *Klebsiella pneumoniae* (7.8 %), followed by *Acinetobacter* species. (6.9 %) (Hariharan *et al.*, 2014). *Acinetobacter* species. have also been reported in dogs with chronic eczema without clinical signs of secondary infection some time ago.

In this study the bacterial isolated from dog fecal sample such as *Escherichia coli* showed varied resistance patterns across different antibiotics, with highest resistance to ceftriaxone (51.3%) and nitrofurantoin (12.8 %). *Acinetobacter baumannii* exhibits high resistance to several antibiotics, with notable resistance to meropenem (100 %) and ofloxacin (94.9 %). *Klebsiella* species display diverse resistance, with highest resistance to Ceftriaxone (66.7 %) and chloramphenicol (71.8 %). High resistance levels in *Acinetobacter baumannii* raise concerns about limited treatment options. Varied resistance patterns highlight the importance of targeted antibiotic use. *Escherichia coli's* resistance to ceftriaxone suggests a need for cautious use of this antibiotic. *Acinetobacter baumannii's* high resistance to meropenem indicates potential challenges in treating infections caused by this bacterium. Rodrigues *et al.* (2002) also reported that dogs and dog faecal matter have been implicated to be carriers of antibiotics resistant bacteria strains (Rodrigues *et al.*, 2002). Similarly, resistance to three or more antibiotics and six or more antibiotics were observed in this current study likewise the report of Cinquepalmi and colleagues (Cinquepalmi *et al.*, 2012) which are in consonance with the present study. Furthermore, a previous study highlighted the antibiotics sensitivity results pattern that could be used for treating enteropathogens in dog faecal matter originated infection, thus with gentamycin being the most sensitive 95 %, followed by azithromycin 50%, enrofloxacin 25 % and cefotaxime 20 %. This is not in agreement with this study since the most sensitive antibiotic was tarivid 96 %, followed by augmentin 86%, gentamycin 80 %, ceporex 78 %, ciprofloxacin 68 %, septrin 36 % and peflacin 18 % (KokilaPriya *et al.*, 2017). This difference in the sensitivity pattern may be as a result of different genes found in these pathogens and the presence of plasmid in their cells. It could also be as a result of excess and indiscriminate consumption of antibiotics most times without physician prescription and monitoring.

Obioma *et al.* (2020) reported that most of the pets are resistant to antibiotics, which pose a general threat to the community thereby putting the public at risk of getting infected. Therefore, it is important that these domestic pets should be treated and vaccinated properly. Also, pets and humans should not be allowed

to eat in the same plate or sleep on the same bed as these served as a major source of transmitting zoonotic infections to man.

SUMMARY OF THE FINDINGS, CONCLUSION AND RECOMMENDATIONS

6.1. Summary of the Findings

The study evaluated the antibiogram patterns of some Gram negative bacteria (*Acinetobacter baumannii*, *Escherichia coli*, and *Klebsiella* species) associated with domestic dog faecal matter in Abakaliki Ebonyi state. A total of Ninety two (92) dogs' faecal samples were collected from four areas which include Democracy Estate Residential (23), Onwe Road Residential (23), Mile 50 Residential (23) and Odinukwe Residential (23). Self- structure questionnaire was used to obtain information from the dogs owners. The samples were analyzed using microbiological standard techniques. The Antibiotic susceptibility pattern of the isolated were determined using Kirby-Bauer disk diffusion methods. From the sample analyzed, 92.3 % of the dog owners use them for security. 72.8 % of the dog owners had only 1 dog while 21.7 % had 2-3 dogs. Dog owners whose dogs were 1 – 6 months were 29.3 % while those with 7 – 10 months were 21.7 %. Owners of German shepherd was 25 % while bull dogs were 20.7 % and Labrador retriever dogs were 32.6 %. However, local dogs were 21.7 %. 50 % vaccinate their dogs once yearly while 37 % and 13 % vaccinate their dogs twice. The bacteria isolated were Gram negative bacteria (*Acinetobacter baumannii*, *Escherichia coli*, and *Klebsiella* specie) with frequency of 12.2 %, 8.2 % and 79.6 %, respectively. 33.3 % *Klebsiella* species were isolated from Mile 50 and Democracy Estate, 16.7 % were isolated from Odinukwe residential area and Onwe Road. However, the *Acinetobacter baumannii*, none were isolated from Onwe Road, 50 % were isolated from democracy estate while 25 % each were isolated from Odinukwe and Mile 50. *Escherichia coli* was the highest bacteria organism isolated representing (39/49) (79.6 %) of all bacteria isolated. Distribution of *Escherichia coli* in different locations showed that 33.3% were from Mile 50, axis 25.6 % were from Odinukwe and Democracy Estate while 15.3 % were isolated from domestic dog fecal samples from Onwe Road. Most antibiotic such as nitrofurantoin (98.9 %) amoxicillin (96.1 %) and

ceftriazone (88.3 %) were resistant to the bacteria isolate from Odinukwe residential, Mile 50 residential, Democracy estate residential and Onwe Road Residential. It is important to implement strict sanitation measures to reduce the environmental impact of bacteria from dog fecal matters. It therefore recommended that dog's owners should properly vaccinate their dogs accordingly.

6.2. Conclusion

The bacterial isolates from dog fecal matter in Abakaliki include potentially pathogenic organisms such as *Acinetobacter baumannii*, *Escherichia coli*, and *Klebsiella* species. *Escherichia coli* is the most prevalent bacterium in dog fecal matter in Abakaliki, followed by *Klebsiella* species and *Acinetobacter baumannii* with a prevalence of 79.6 %, 12.2 % and 8.2 % respectively. Mile 50 Residential Area has the highest frequency of *Klebsiella* species (33.3 %) and *Escherichia coli* (33.3 %). *Acinetobacter baumannii* has the highest frequency (50.0 %) in Democracy estate residential area. Odinukwe residential area has relatively lower frequencies for all three bacteria. The result of the study also revealed that nitrofurantoin, ciprofloxacin, ofloxacin, perfloxacin, meropenem, gentamicin, ciprofloxacin and perfloxacin were resistant to the bacteria isolate from Odinukwe residential, Mile 50 residential, Democracy estate residential and Onwe Road Residential.

6.3. Recommendations

1. Sanitation Measures: it is important to implement strict sanitation measures to reduce the environmental impact of *Escherichia coli* from dog feces.
2. Pet Owner Education: Dogs owner should be educated about proper waste disposal practices to minimize the spread of bacteria.
3. Monitoring and Surveillance: Establish continuous monitoring and surveillance to track changes in bacterial frequencies and assess the effectiveness of interventions are essential.
4. Vaccination: dogs owners should properly vaccinate their dogs accordingly

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