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Research Article

Comparism of Different Antibiotic Brands and Antibiogram of Some Bacteria Isolated from Dogs in Environmentally Challenged Communties of Eleme LGA of Rivers State

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ABSTRACT

Threat of antibiotic resistant pathogens has become serious global public health issue, leading to emergence of different brands of antibiotics from different pharmaceuticals. This study was aimed at comparing and evaluating different brands of antibiotics on pathogenic bacteria isolated from domestic dogs in some environmentally challenged communities in Eleme. A convenience sampling technique was used to aseptically collect fifty (50) samples from the mouth and nostrils of domestic dogs. Culture, isolation and identification of organisms were explored using standard microbiological techniques. Different brands of antibiotic sensitivity discs were purchased from pharmaceutical retail outlets and sensitivity testing performed as described by the Clinical Laboratory Standard Institute (CLSI). Bacteria isolated and identified and frquency were *Escherichia coli* (21%), *Klebsiella spp* (20%) and *Proteus spp* (7%), *Bacillus spp* (2%), *Staphylococcus aureus* (10%) and unicellular fungus, *Candida albicans* (8%). Three brands of the different antibiotics used were statistically significant at $P>0.05$, Abtek biological limited (71.76(32%), Cetech Diagnostic (72.14(33%), and Oxoid standard single disc (73.06(33%) while locally manufactured disc 1 (4.93 (2%) and disc 2 (9.86(4%) were not statistically significant. There was significant difference between Abtek biological limited, Cetech Diagnostic and Oxoid standard single disc ($P<0.05$). There was no significant difference between locally made discs in comparison to the other three brands. The findings revealed that, common pathogens are becoming increasingly resistant to some locally manufactured antibiotics. Hence there is urgent need to improve various pharmaceutical stewardship which would in-turn improve on the potency of products manufactured.

Keywords: Antibiotic brands, antibiotics, bacteria, domestic dogs, comparism, resistance.

Introduction

The challenge of antibiotic resistance remains a great threat to a progressive healthcare, food production and ultimately life expectancy worldwide. The increasing trend of resistance is attributed to extensive used of antimicrobial agents in human and animal medicine with unethical practices of man in his immediate environment (Marchetti *et al*., 2021).

Hence, these have been known globally to have severe consequence of microbial modification and negative health impact (Wanda, 2018).

Antimicrobial susceptibility testing is an essential tool for drug discovery, epidemiology and prediction of therapeutic outcome. However, with current impact and considerable treatment failure associated with multidrug-resistant pathogen, it has become a serious global public health concern (Balouiri *et al*., 2015).

Dogs are reservoir of diverse pathogenic bacteria and due to population surge in the number of domestic dogs serving as pets and security purposes by many homes, there is an enhanced and increasing interaction between dog and man which have consequently led to potential risk of zoonotic diseases in our communities (Azuonwu *et al*., 2020).

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These diseases could be transmitted anthropozoonotically through infected aerosols, saliva, infected faecal matter, or urine. Nevertheless, several reports have asserted that methicillin resistant *Staphylococcus aureus* (MRSA) are most common pathogenic bacteria associated with dogs which are anthropozoonotically transmitted (Azuonwu *et al*., 2023). Furthermore, other bacterial pathogens include *Leptospira*, *Brucella*, *Salmonella*, *Pasteurella*, *Staphylococcus intermedius*. They could also result to fungal infection e.g dermatophytosis, and parasitic infection e.g toxoplasmosis which is a common zoonotic infection transmitted to humans (Chomel, 2014; Ghasemzadeh and Namzi, 2015).

Antibiotics are important antimicrobial medication essential for the prevention and treatment of bacterial infections. Their basic modes of action include killing or inhibiting bacteria proliferation (Hugo and Russell, 2004). The discovery of antibiotics many years ago to the practice of medicine has contributed immensely to the treatment of many life-threatening infectious diseases. For many decades, different types of antibiotics have been in used. However, extensive usage has led to the emergence of multiple resistant and it is spreading rapidly among the existing bacteria (WHO, 2014). It is reported that there is a significant rise in trends of amoxicillin, co-amoxiclav and streptomycin resistant in *Escherichia coli*, erythromycin resistant in *Staphylococcus* species from samples obtained from clinical cases in small animal hospital and an equivocal rise in trend of *Staphylococcus* species to cephalexin (Normand *et al.*, 2000). A significant increasing resistance of *Staphylococcus intermedius* was observed from isolates obtained from dogs to Penicillin, Neomycin, Erythromycin, Sulphonamide and Co-trimazole (Wissing *et al*., 2001). This is an indication that from time to time, changes could occur in microbial susceptibility and diversity.

The body and gastrointestinal tract of humans and domestic dogs are made of lots of non-pathogenic microbes collectively called commensals or microbiota, including *Staphylococcus aureus*. However, in environmentally challenged communities in the Niger Delta region, Nigeria, where environmental degradation has been on increase partly due to artisanal refining, there is an extended and enhanced degree of pollution of soil, air and water. Therefore, the consequences of such consistent exposure to air pollutants as observed in the environmentally challenged communities might cause dysbiosis of gut microbiome of both humans and domestic animals such as dogs. Moreover, petroleum pollutants have been reported to change the composition and function of soil microbial communities due to their long-term toxic effects on the environment and their resistant to biodegradation (Hassanshahian *et al*., 2012). Consequently, these conditions couple with poor sanitation and lopsided social amenities has immensely exposed the teeming population to diverse pathogenic microorganisms and their novel strains. Communities and villages lack portable drinking water, while some depend solely on water from the streams that are highly polluted with crude oil. Resistant pathogens that have undergone changes over time are also ingested along. Others used atmospheric rain that is highly contaminated with hydrocarbon from artisanal refineries and resistant microbes. Thus, for most commensals and other pathogenic bacteria to survive in such vicinity, they undergo microbial modification (Hassanshahian *et al*., 2012). The common microbiota and pathogenic microbes could undergo changes with resistant genes, hence rendering common and conventional countermeasures ineffective. Studies have reported a continuing change in prevalence and epidemiology of pathogenic bacteria with new strains being discovered from mouth, nose, ears, and faecal matter of dogs (Azuonwu *et al*., 2023; Ibira *et al*., 2023).

Thus, an increased awareness for MRSA and other novel strains of pathogenic bacteria through robust investigation of host specific transmission route and characterization in environmentally challenged communities is highly essential. Africa and Nigeria specifically lack comprehensive and adequate support system for MRSA colonization in domestic dogs in correlation with anti-biogram susceptibility of different strains of the organism. Nonetheless, different brands of antibiotics manufactured by many pharmaceutical industries are ineffective against many microorganisms hence, instead of inhibition or killing, they induce resistance.

Therefore, the present study was designed to determine the activity of the different antibiotic brands and antibiogram profile of some bacteria isolated from dogs in an environmentally challenged communties of

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Eleme, in Rivers State in the Niger Delta. It is therefore, strongly expected that data generated would stimulate stronger evidence base research and conversation for prompt diagnosis and management of the disease outcome from demostic dogs in the Niger Delta region, given the paucity of research information in this direction.

Materials and Methods

Description of the study area

This study was carried out on samples collected from domestic dogs in Eleme Local Government Area of Rivers State in the Niger Delta in Southern Nigeria. Eleme lies in greater Port Harcourt metropolis with headquarters in Ogale covering 138 km^2 and located in latitude 5^o 04'60.00" N and longitude 6^o 38'59.99"E in Rivers State, Niger Delta region (Oyebade and Buochuama, 2016). The Map of the study area is shown in Figure 1.

The Niger Delta Region is rich in rainforest and mangrove swamp with rich fertile soil endowed with crude oil. Eleme is an industrialized town and manufacturing hub including two crude oil refineries, a fertilizer company and petro-chemical and other business activities such as trading and farming. Some communities situated in Eleme are Aleto, Agbonchia, Ogale, Alode, Ebubu, Ajamas, Ibuluya, Yaa, Eteo, and others as shown in Figure 1.

Antibiotics selection

Four different antibiotics (Ciprofloxacin, Gentamycin, Cefuroxime, and Amoxicillin) products of five (5) different antibiotic brands were purchased from Pharmaceutical retail outlets for this study. The brands comprised two (2) locally produced, two (2) foreign, and one (1) foreign standard disc. The brands were Abtek Biological Limited (Liverpool), Cetech Diagnostic, (Belgium), locally manufactured disc 1 and disc 2 (Nigeria), Oxoid Standard Single Disc (London).

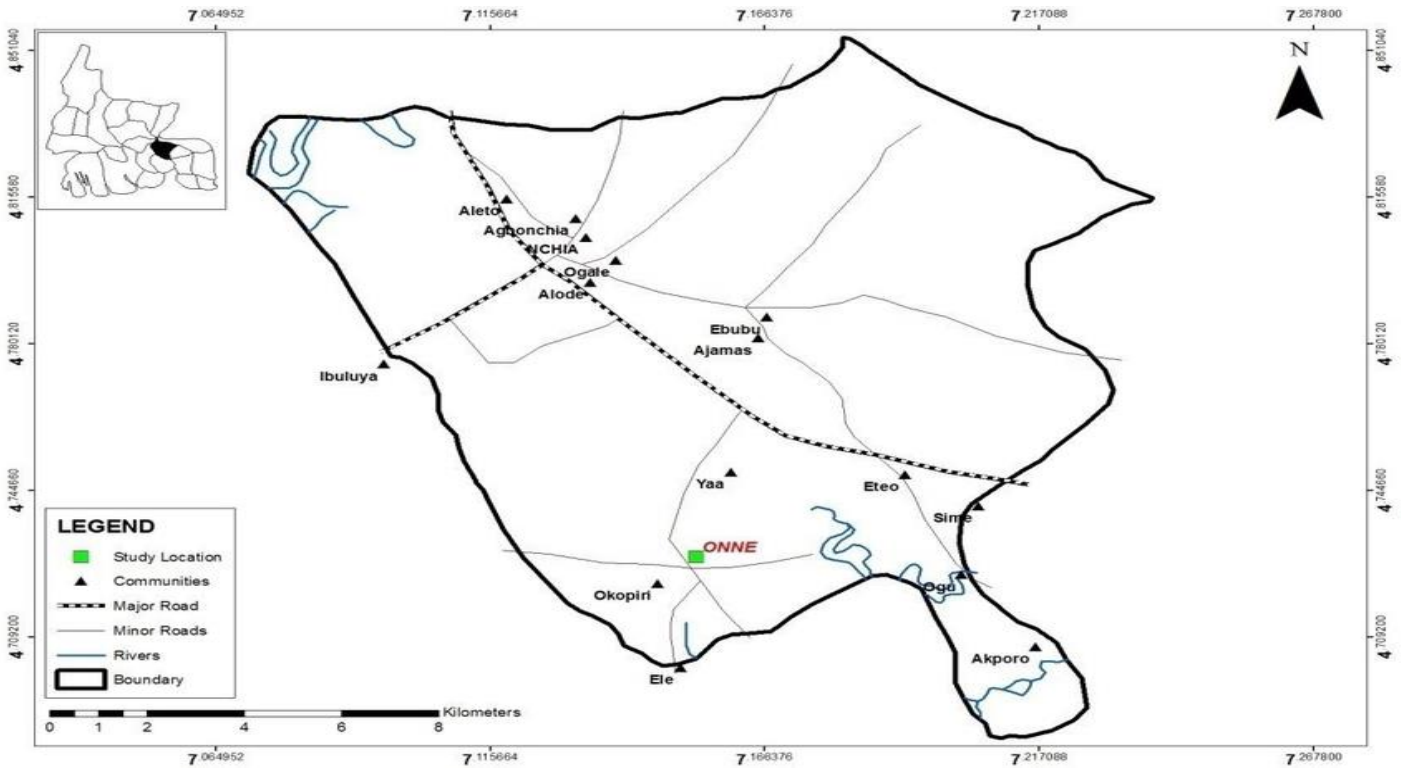

Figure 1: Map of the study area, showing different communities in Eleme, Rivers State
(Adapted from Oyebade and Buochuama, 2016)

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Sample collection and processing

A total of 50 samples were collected from the mouth and nostrils of domestic dogs in different locations and were transported immediately in a cold ice pack to Medical Microbiology Department, University of Port Harcourt Teaching Hospital for laboratory analyses. Sample aliquots were aseptically cultured on Nutrient, MacConkey, Chocolate, and Blood agar accordingly. All standard procedures for bacterial culture as described by Cheesbrough (2006) and Clinical Laboratory Standard Institute (CLSI) were strictly followed. The culture preparations were incubated at a temperature of 37°C for 24 hours to produce discrete colonies.

Isolation and identification of bacteria

Distinct colonies were collected and sub-cultured on separate media. Gram staining and biochemical tests such as coagulase, citrate, indole, oxidase, urease, other carbohydrate tests as well as microscopy were explored appropriately to identify and classify the pathogens isolated as described by Cheesbrough (2006).

Antimicrobial susceptibility assay

Kirby-Bauer method (disc method) was explored as described by Cheesbrough (2006). Pure culture plates of each isolated organism were selected and its colony aseptically emulsified in a sterile saline solution and was mixed thoroughly to ensure that no solid material from the colony is visible in the saline solution. It was repeated until turbidity of the saline solution virtually matched that of the standard turbidity. A standard wire loop was used to collect the organism inside the tube and streaked completely on Mueller-Hinton agar (MHA) plate. It was allowed for 5 minutes to dry before antibiotic discs were placed on the surface of the agar using sterile forceps. The disc was gently pressed onto the surface of the agar using flamed sterilized forceps. The inoculated plate was gently inverted and incubated for 24 hours at a temperature of 37^0C. After incubation, a metric ruler was used to measure the diameter of the zone of inhibition for the antibiotics used. The measurement obtained from the individual antibiotic to the standard table determined whether the tested bacterial species is sensitive or resistance to the tested antibiotic respectively.

The results were categorized based on their standard range and grouped into sensitive, intermediate, and non-sensitive as described in Clinical Laboratory Standard Institute (CLSI)

Statistical Analysis

Data obtained were collated using Microsoft excel and transferred into Statistical Package for Social Science (SPSS) version 21 for analysis. The parameters include mean, standard deviation percentage, ANOVA and correlation. The significance was observed at probability level of less than or equal to 0.05. Results were presented in figures, tables, and charts respectively.

Results

Results of the prevalence of each organism isolated from the dogs and expressed in percentages are shown in Figure 2. The pathogens were gram negative rods, which are *Escherichia coli* (21%), *Klebsiella spp* (20%), *and Proteus spp* (7%), gram positive rod which is *Bacillus spp* (2%), gram positive cocci which is *Staphylococcus aureus* (10%) and a unicellular fungus, *Candida albicans* (8%).

Table 1 shows the mean values (mm) of each antibiotic brand in comparison. Abtek Biological Limited (Liverpool) showed mean value of 23.20, 1.33, 22.44 and 24.76 for Amoxicillin, Cefuroxime, Gentamycin and ciprofloxacin respectively. Cetech Diagnostic, (Belgium) showed mean value of 22.10, 1.34, 23.85, and 24.87 for Amoxicillin, Cefuroxime, Gentamycin and ciprofloxacin respectively. The Locally produced disc 1 has mean value of 1.23, 1.13, 1.12 and 1.44 for Amoxicillin, Cefuroxime, Gentamycin and ciprofloxacin respectively. Locally made disc 2 has mean value of 2.44, 2.24, 2.25, and 2.86 for Amoxicillin, Cefuroxime, Gentamycin and ciprofloxacin respectively, the oxoid standard single disc made in London had mean value of 22.39, 1.36, 22.55 and 26.78 for Amoxicillin, Cefuroxime, Gentamycin and ciprofloxacin respectively.

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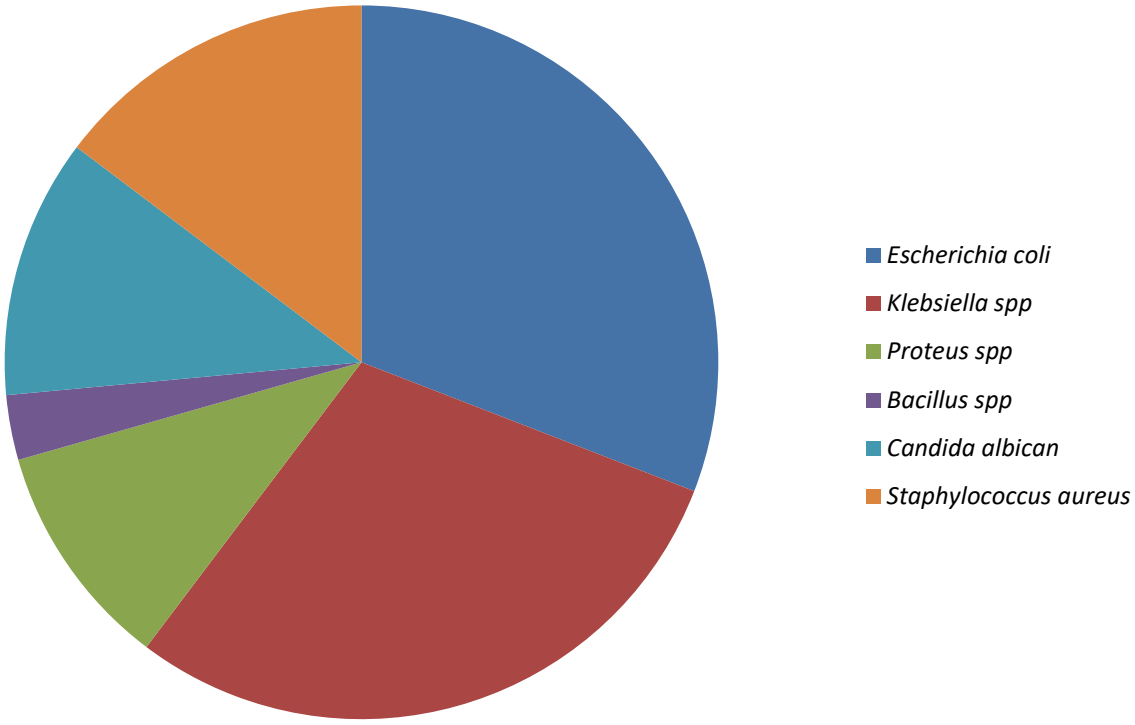

Figure 2: Prevalence of bacterial isolates and *Candida albicans* from domestic dogs.
Note: The distribution of the microbes in percentages are *Escherichia coli* (21%); *Klebsiella* spp (20%); *Proteus* spp (7%); *Bacillus* spp (2%); *Candida albicans* (8%); *Staphylococcus aureus* (10%).

Table 1: Mean Comparison of the activity of Different Brands of Antibiotic Disc and Selected Antibiotics

Antibiotic Brands	AM	CE	GE	CI	Summation	t-value	DF	*p*-value	Remark
Abtek Biological Ltd., Liverpool	23.20	1.330	22.44	24.76	71.76(32%)	5.4067	68	0.02	Sig.
Cetech Diagnostic, Belgium inc	22.10	1.340	23.84	24.86	72.14(33%)	2.0843	68	0.03	Sig.
Locally Made Disc 1	1.233	1.134	1.123	1.444	4.93(2%)	0.2408	68	0.88	N/S
Locally Made Disc 2	2.46	2.26	2.24	2.86	9.86(4%)	0.4816	68	0.89	N/S
Oxoid, London Standard Single Disc	22.38	1.36	22.54	26.78	73.06(33%)	6.4341	68	0.02	Sig.

Key: Non-significant, Sig.= Significant, DF=Degree of freedom, AM=Amoxicillin, CE=Cefuroxime, GE=Gentamycin, CI=Ciprofloxacin

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Table 2 shows the mean comparison of total heterotrophic count (THC) and antibiogram pattern of samples that were collected from each sites.

The value for THC were 295.514±314.410 for mouth swab and 233.45±246.319 for nasal swab, Amoxicillin was 21.20±8.700 for mouth swab and 19.91±8.680 for nose swab, Cefuroxime was 1.43±4.937 for mouth swab and 0.30±1.741 for nose swab, Gentamycin with a value of 22.94±8.657 for mouth swab and 22.15±9.602 for nose swab and ciprofloxacin was 25.66±8.314 for mouth swap and 24.06±7.433 for nose swab.

The activities of each antibiotic were also compared. Figure 3 showed that Ciprofloxacin and Gentamycin have the highest degree of activity for all the different brands of antibiotics selected except for the locally made with low antibiotic activities. Amoxicillin and Cefuroxime showed lower activities for the entire brands as demonstrated in the bar chart.

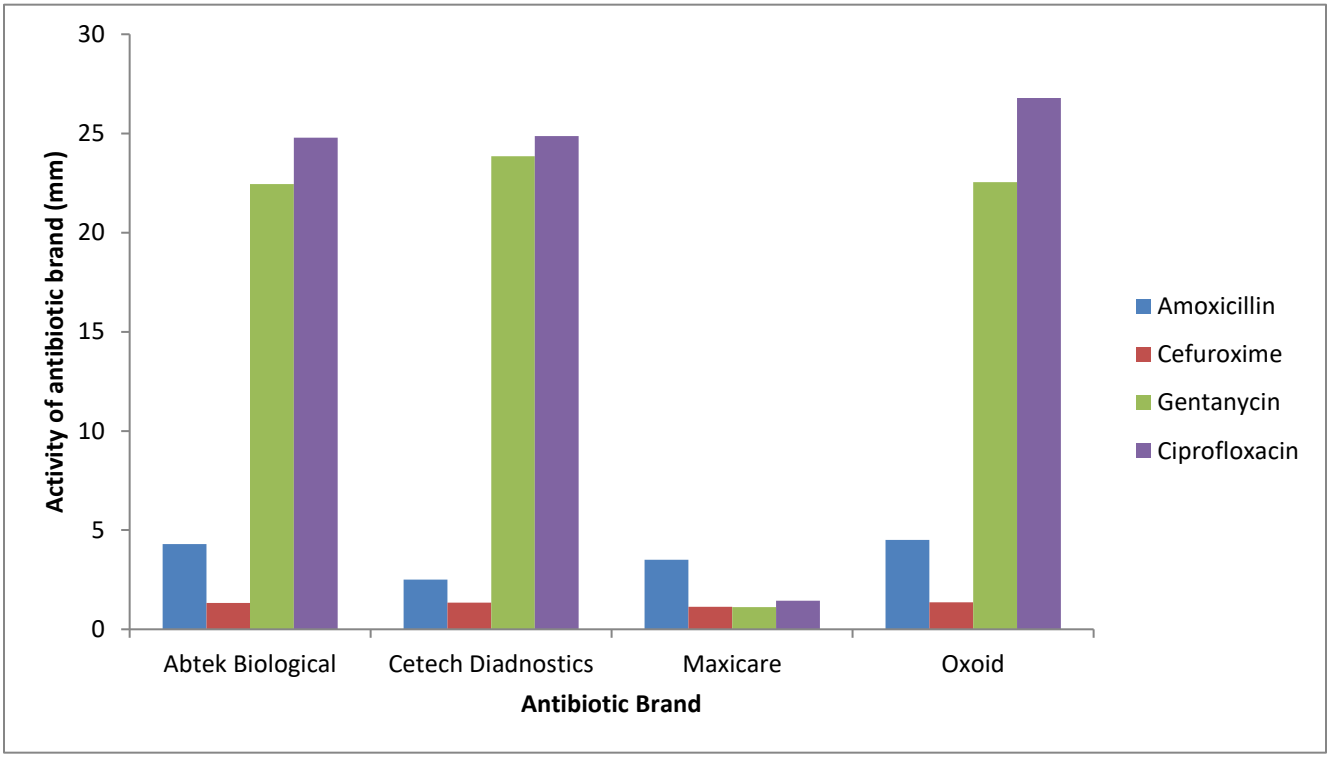

Figure 3: Comparison of the activity of different antibiotic brands and selected antibiotics

Table 2: Mean values of THC and antibiotic sensitivity pattern of mouth swap with nasal swab isolates

Parameter	Mouth Swab (N=27) Mean±SD	Nasal Swab (N=23) Mean±SD	t-value	DF	p-value	Remark
THC	295.51±314.410	233.45±246.319	0.9024	66	0.37	Significant
Amoxicillin	21.20±8.700	19.91±8.680	0.6118	66	0.54	N/S
Cefuroxime	1.43±4.937	0.30±1.741	1.2435	66	0.22	Significant
Gentamycin	22.94±8.657	22.15±9.602	0.3567	66	0.72	N/S
Ciprofloxacin	25.66±8.314	24.06±7.433	0.8348	66	0.41	Significant
Control	23.20±8.185	23.30±8.755	0.0487	66	0.9	N/S

Key: N/S = Non Significant, THC= Total Heterotrophic Count

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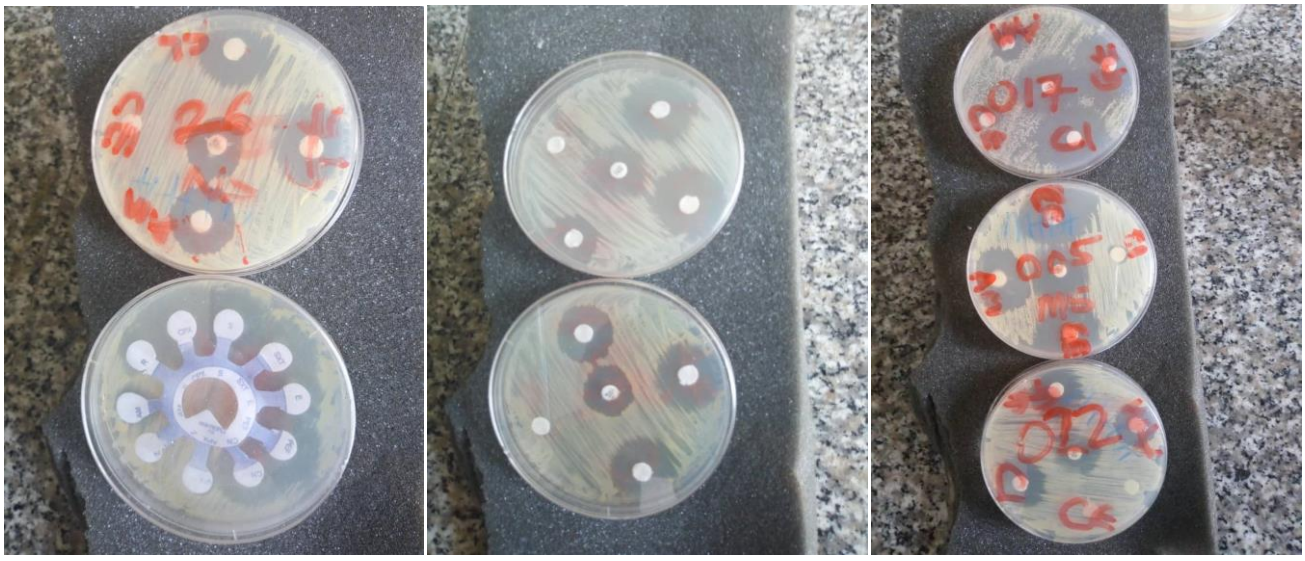

Plate 1: Some antibiotic sensitivity assay of isolates of the study

Discussion

The result obtained from samples collected from domestic dogs yielded positive outcome of different pathogens. Figure 1 described the prevalence in percentages of various pathogenic bacteria isolated from the mouth and nose of domestic dogs in Eleme local government area. The pathogens were *Escherichia coli* (21%), *Klebsiella* spp (20%), *Proteus* spp (7%), *Bacillus spp* (2%), *Staphylococcus aureus* (10%) and *Candida albicans* (8%). This result is in congruent with work done by Kasempimolporn *et al*. (2001) and Luca *et al*. (2004) who recovered similar pathogen from the same sites of pet dogs except the variation in prevalence which could be due to difference in dog breed and geographical location. Azuonwu *et al*. (2020 and 2023) also reported the similar pathogens from the same site with similar prevalence.

However, other researchers reported additional pathogens, *Streptococci* and *Pseudomonas* which were not found and isolated from this study. This could be due to diversity in geographical location/population and anatomical sites where samples were obtained. Moreover, *Campylobacter, Salmonella*, *Brucella* and many other pathogens have been reported and found to be most common bacterial pathogens in pet dogs by researchers (Mark, 2003; Zahra, 2007, Earnest *et al*., 2018; Scott, 2018).

In this current study, the bacterial pathogens isolated and their prevalence were; *Escherichia coli* (21%), *Klebsiella* spp (20%), *Bacillus* spp (2%), and *Proteus* spp (7%), which had the least prevalence. This finding has significant public health implication from the stand point that domestic dogs carry pathogenic bacteria with lethal potentials to cause zoonotic infection to the residents of affected communities.

Currently, most bacterial infections have promising treatment outcomes due to availability of various brands of antibiotics. However, the proliferations of antibiotic resistant pathogens have raised issues of serious public health concern globally in recent time. Addressing this threat requires a continuous aggressive action to preventing the spread of novel species of microorganisms.

Table 1 and Figure 2 showed and demonstrated the mean comparison of different antibiotic brands on microorganism isolated from environmentally challenged community of Eleme. The researchers finding revealed the effectiveness of different brands of antibiotics as used in this study. Abtek Biological Limited (Liverpool), Cetech Diagnostic (Belgium) and Oxoid Standard Single Disc (London) were all very effective for Ciprofloxacin, Gentamycin, and Amoxicillin except for Cefuroxime, which was highly resistant.

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The resistance in Cefuroxime could be adduced that the organisms might have undergone mutational modifications which impacted on the effectiveness of the antibiotic or it could be that the antibiotic has been routinely deployed in veterinary medicine for pets such as domestic dogs and consequently abused over time.

Contrastingly, the high sensitivity of Ciprofloxacin and Gentamycin could be that these antibiotics have not been abused among pets.The locally made disc 1 and 2 were highly insignificant with only 2% and 4% effectiveness respectively on the organisms isolated. In similar report by Ibira *et al*. (2023), Amoxicillin was reported to have antibiogram activity rate of 11.90%, while Ciprofloxacin had 52.38%, though the brands were not specified in their report. Also, finding in this study revealed higher antibiogram rate of both Amoxicillin and Ciprofloxacin for the different category except for locally made disc 1 and 2 brand. The inactivity observed from the locally made disc 1 and 2 brand could be due to emergence of novel species of pathogens which may have probably developed resistance over time to the brands. In a related study conducted by Azuonwu *et al.* (2020), which corroborates with this study, Ofloxacin was reported as the most sensitive antibiotic with (96.0%) susceptibility rate followed by Augmentin (86.0%), Gentamycin (80.0%), Ceporex (78.0%), Ciprofloxacin (68.0%), Septrin (36.0%), while Pefloxacin was the least sensitive with (18.0%). Although statistical analysis shows that there is no significant difference in zones of inhibition between Abtek Biological Limited (Liverpool), Cetech Diagnostic (Belgium) and Oxoid Standard Single except locally made disc 1 and 2 antibiotic brand. However,a similar study conducted by Nazir and colleagues (2009), a significant different was reported between the Oxoid standard single discand other brands of antibiotics used in their study. Although the antibiotic brand used in their study were Cyrocin (Highnoon Laboratories Limited), Ciproxin (Bayer Pharma (Pvt) Ltd.-Pakistan), Mercip Merck Marker (Pvt.) Ltd., Pakistan) and Axcin (Sandoz - Norvatis Pharma Ltd., Pakistan) which could be the cause of the variation.

Domestic dogs' oral and nasal cavity carries potential pathogens capable of increasing the burden of antibiotic resistance. Table 2 showed the mean comparison of the total heterotrophic count and antibiogram pattern of antibiotics on mouth swab and nasal swab isolates.

The current study has demonstrated that, there is no significant difference between mouth swabs and nasal swabs total heterotrophic count. This implies that, there is no significant difference between the different antibiogram patterns of the antibiotic brands on the isolates from each site. Ibira *et al*. (2023) and Azuonwu *et al.* (2023) have also given the same verdict on samples collected from the same sites. This suggests that similar strains of bacterial pathogens could be recovered from the buccal and nasal cavity of dogs and could consequently result to community health challenges.

In conclusion, the study finding revealed the effectiveness of different brands of antibiotics as used in this study. Abtek Biological Limited (Liverpool), Cetech Diagnostic (Belgium) and Oxoid Standard Single Disc (London) were all very effective for Ciprofloxacin, Gentamycin, and Amoxicillin except for Cefuroxime, which was highly resistant. The resistance in Cefuroxime could be adduced that the organisms may have undergone mutational modifications which impacted on the effectiveness of the antibiotic or it could be that the antibiotic has been routinely deployed in veterinary medicine for pets, such as domestic dogs and consequently had been probably abused over time. Nevertheless, the common bacterial pathogens are becoming increasingly resistant to some first line antibiotics products manufactured locally.

Thus, with an already weak health system and an under quality antibiotic products which are being produced daily, there is need for standard policies and procedures, which should be streamlined for various pharmaceutical manufacturing sectors, so as to improve on the potency and quality of the products manufactured for the overall benefit of both human and animals wellbeing in our communities

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