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Full Length Research Article

ORAL CAVITIES MULTIDRUG RESISTANT BACTERIA COLONIZATION IN APPARENTLY HEALTHY DOGS IN JOS, PLATEAU STATE, NIGERIA

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ABSTRACT
Dogs harbor pathogenic, zoonotic or multidrug resistant bacteria in their oral cavities and may serve as a possible source of transmission to humans through direct contact or bite. We therefore carried out a clinic-based cross-sectional study design to assess the level of multidrug resistant bacteria colonization of oral cavities of apparently healthy dogs presented for routine examination, vaccination and deworming. Oral swabs were taken from 100 apparently healthy dogs of different age, breed and sex. Isolation and identification of bacteria was done based on colony morphology and biochemical test. Antibiotic sensitivity test to 12 antibiotics was carried out on the isolates using Kirby-Bauer disk diffusion method according to standard protocol. The study showed that the oral cavities of all the dogs included in the study have one or more bacteria species. *E. coli* accounted for the greater proportion (43.21%) of the isolates. Other isolates include *Klebsiella* spp, *Bacillus* spp and *Staphylococcus aureus*. There was also a mixed isolates of *E. coli* and *Yeast* (23.57%), *E. coli* and *Bacillus* (3.57%), *E. coli* and *Staphylococcus* (1.79%), *E. coli* and *Streptococcus* (1.79%) and *Staphylococcus* and *Bacillus* (1.79%). There was no statistically significant difference in the isolation based dogs' characteristics such as age, sex, breed and management system. Varying degree of sensitivity was observed in the isolates. Some of the isolates displayed resistance to 2 or more antibiotics. The isolates showed resistant phenotype to β-lactam antibiotics-amoxicillin and ampicillin ranging between 75.0%−100.0% and 0.0%−83.0% respectively. Susceptibility of the isolates ranges between 83.3%−100.0%, 66.7%−100.0%, 63.7%-100% and 50.0%−83.3% for fluoroquinolone antibiotics-peflacin, ciprofloxacin and nalidixic acid and aminoglycoside-streptomycin respectively. The dogs' population sampled during the period were 6 years old and below. Six breeds of dogs; Caucasians, German shepherd, mastiff, Rottweiler and indigenous breeds were sampled. Apparently healthy dogs of different ages, sex, breeds, and management systems are colonized with multidrug resistant bacteria in their oral cavities and suggest a possible transmission to their owners and or handlers. Indiscriminate antibiotic use should be avoided by dog owners. Culture and antibacterial sensitivity testing in the event of dog bite is recommended.

Keywords: Antibiotic, bacteria, dog, isolation, resistance

INTRODUCTION

The dog as one of mans' closest companion animal lives in close relationship with man and share the same home environment, eat animal food products and in most cases is treated with antimicrobials used for humans (Murphy *et al*., 2009). In the United States, it has been estimated that over 52 million dogs live in the same environment with humans (Karla and Abiodun, 2008). Though there is no available data, the situation may not be so much different in Africa as increase in human population is met with attendant increase in dog population (Gascoyne, 1994).

Wandeler *et al*. (1993) showed that among many reasons, culture, status, social interests, religious convictions, and economic activities mostly form the basis for which domestic dogs are kept by humans. The relevance of dog keeping has been shown to cut across all categories of people irrespective of socioeconomic background and geographical location (Westgarth *et al*., 2007).

Dogs harbor or are colonized by bacteria organisms that may not be pathogenic to them. Due to their close relationship with man, they may serve as reservoir of infection to humans (Lefebvre *et al*., 2009). Of the bacteria colonizing oral cavities of dogs, some are pathogenic, zoonotic or carrying antibiotic resistant gene. Humans usually acquire pathogenic, antimicrobial resistant, and or zoonotic bacteria from dogs through contact.

According to Abrahamian and Goldstein, 2011, dog keeping constitutes a significant hazard due to their bite which is the most common form of skin injury encountered in man. The bite of dogs serves as an important entry of bacterial contaminants leading to wound infections (Goldstein, 1992). Multidrug-resistant bacteria of public health importance have been found in people and companion animals (Lefebvre *et al*., 2009; Weese *et al*., 2010). Studies have shown that pet owners have more likely hood and greater risk of colonization with extended-spectrum β-lactamase–producing *Escherichia coli* than people who do not own pets (Meyer *et al*., 2012). *Staphylococcus aureus* is one of the most common pathogens among humans and animals (Petinaki and Spiliopoulou, 2015). *S. aureus* carries on the chromosome several genomic islands, including antibiotic resistance determinants and virulence genes (Hiramatsu *et al*., 2013)

Studies have implicated bacteria flora found in dogs' oral cavity to be the major cause of bite wound infection (Allaker *et al.,* 1997; Forsblom *et al.,* 2004; Abrahamian and Goldstein, 2011; Kirketerp *et al*., 2011). The knowledge of the microbial flora of the oral cavity of dogs may therefore be useful in predicting the likely bacteria to be found in infected bite wounds inflicted by dogs.

We undertook this survey to investigate for common species of bacteria in dogs' oral cavity and to determine their resistance to some antibiotics to form a baseline that will be useful in predicting the likely bacteria to be found in infected bite wounds inflicted by dogs and the antibiotics that could be used. It will also be useful in understanding the role of dogs in co-transmission of certain species of bacteria to the human family due to their close association.

MATERIALS AND METHODS

Study Area

The study was conducted in Jos, Plateau State Nigeria. Jos is located at latitude: 10° 0' 0 N, longitude: 9° 30' ^{0} E on the Jos Plateau (Wikipedia, 2012). The city is divided into three separate Local Government Areas: Jos-North, Jos-South, and Jos- East with a combined population density of 391 persons per Km^2. The city has an altitude of 1,217 m above sea level and so enjoys a more temperate climate than most of the rest of Nigeria (Plateau State (n.d). Retrieved November, 29 2018 from https://en.wikipedia.org/wikis/Plateau _state). Though the city is situated in the tropical zone, because of a higher altitude it has a near temperate climate with an average temperature of between 18 and 22°C. Harmattan winds cause the coldest weather between December and February. The warmest temperatures usually occur in the dry season months of March and April. The mean annual rainfall is 146 cm. The highest rainfall is recorded during the wet season months of July and August. Plateau State in Nigeria has a large population of dogs due to the cultural acceptance as meat as well as good weather condition for keeping of exotic breeds (Bata *et al*., 2011).

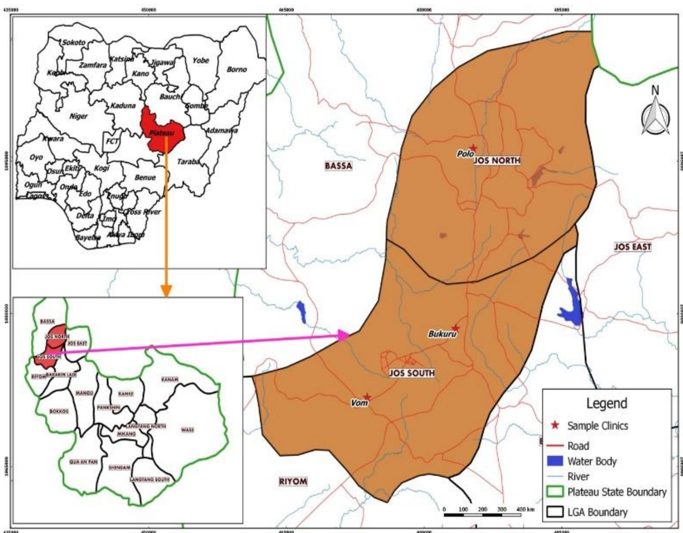

Fig 1: Map of Nigeria showing the study area

Study Design

A clinic-based cross-sectional study design was carried out between December, 2017 to July, 2018. The veterinary clinics identified were included in the study based on the number of cases presented in a week. Those with a minimum of 100 cases were included. These include the ECWA Veterinary Clinic, Bukuru, the Federal College of Animal Health and Production Technology, NVRI Vom vet clinic and the Plateau State veterinary hospital, Polo. The purpose of the study was first explained to dog owners and their consent obtained. Participation was voluntary and only apparently healthy dogs presented for routine deworming and vaccination were included. Animals presented with complains of off feed, vomiting and diarrhea were not included in the study

Sample collection and handling

Oral swabs were collected from individual dogs presented for routine clinical examination, vaccination and or deworming in some veterinary clinics in Jos, Plateau State. Characteristics such as age, sex, breed and the management system of each dog were recorded. The samples were labeled and transported on ice to the Diagnostic laboratory, National Veterinary Research Institute Vom for microbiological analysis.

Culture and Isolation of bacteria

Culture and the isolation of bacteria were carried out as described by Cheesbrough (2006) and modified by Awoyomi and Ojo (2015). Each sample was subjected to nonselective pre-enrichment in 9 ml of Tryptic Soy Broth (TSB) for 6 to 8 hours at 37^{O}C. The TSB culture was inoculated onto 5% blood agar (Oxoid CM0271 ® Basingstoke, UK) and MacConkey agar (Oxoid CM0115 ®Basingstoke, UK) plates. Inoculated plates were incubated aerobically at 37^{O}C for 24 to 48 hours. After incubation, plates were examined for bacterial colonies. Isolates were identified by colonial morphology, microscopy (following Gram's staining) and biochemical characterization. The biochemical tests included oxidase, catalase starch hydrolysis, casein hydrolysis, indole test, methyl-red and voges-proskauer test (MR-VP).

Antimicrobial susceptibility testing

The identified isolates were subjected to antimicrobial susceptibility tests using the disc diffusion techniques as described by Bauer *et al.,* (1966) and according to the guidelines of the Clinical Laboratory Standard Institute (CLSI, 2008). Isolated bacterial colonies were mixed in normal saline and turbidity was matched with 0.5 McFarland turbidity standards. The antibiotic discs (Oxoid, UK) were evenly dispensed unto the surface of the inoculated Mueller-Hinton agar plate using a disc dispenser and were gently pressed down to ensure complete contact with the agar surface. The plates were inverted and incubated at 37°C for 18 h. The diameter of zones of inhibition observed was measured with a ruler and compared with a zone interpretation chart (Muragkar *et al*., 2004). McFarlland scale was used to classify isolates as sensitive, intermediate or resistant. The concentrations of the antibiotics used are: Nalidixic acid (30 µg), peflacin (10µg), gentamycin (10 µg), augumetin (30µg), ciprofloxacin (10µg), septrin (30µg), streptomycin (30 µg), ampicillin (30 µg), ceporex (10µg), tarivid (10µg), and amoxicillin (30µg).

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DATA ANALYSIS
Descriptive statistics (frequencies, simple percentages) was used to present the data obtained.

RESULTS
Dog's profile
Greater proportions 46/100(46.00%) of the dogs sampled were of <1year, 34/100 (34.00%) were of 1-2 years, while, 20/100 (20.00%) were >2years. Based on breeds, 24/100(24.00%) were German shepherd, 4/100 (4.00%) mastiff, 2/100 (2.00%) Rottweiler, 30/100 were Caucasians, 40/100 (40.00%) local dogs. In all Greater proportion, 60/1000(60.0%) were exotic breeds and 40/100 (40.0%) were Local breeds. Of the 100 dogs sampled; 54 are female and 46 males. Based on management practice, 62 are managed intensively, 24 semi-intensive and 14 extensively (Table1).

Bacteria isolated from the oral cavity of dogs
Bacteria were isolated from all (100.0%) of the 100 samples collected (Table 2). Of the bacteria isolated, greater proportions were *E. coli* (43.21%), followed by *Klebsiella* spp and *Bacillus* (8.93%) and least is *Staphylococcus* spp. Others were mixed isolates of *E. coli* and *Yeast* (23.57%), *E. coli* and *Bacillus* (3.57%), *E. coli* and *Staphylococcus* (1.79%), *E. coli* and *Streptococcus* (1.79%) and *Staphylococcus* and *Bacillus* (1.79%) (Table 2).

Antibiotic sensitivity pattern of bacteria isolated from the oral cavity of dogs
The result showed that all the bacteria were resistant to 2 or more antibiotic suggesting multi-drug resistance. Resistance of the isolates to amoxicillin and ampicillin ranges between 75.0%−100.0% and 0.0%−83.0% respectively across the isolates. The *E. coli* isolates were more susceptible to peflacin (94.1%), followed by Tarivid (88.2%) and the least is amoxicillin. The result also showed that the *Klebsiella* isolates were more susceptible to peflacin (83.3%), streptomycin (83.3%) and ciprofloxacin (83.3%) while the *Bacillus* species were more susceptible to Augumentin (83.3%) followed by peflacin. *Staphylococcus* isolates were highly susceptible to ciprofloxacin and peflacin (100.0%). This suggests that all the isolates were susceptible to peflacin at high proportion.

Table 1. Characteristics of dogs sampled

Variable	Frequency	Percentage %
Age		
0-1 year	46	46.00
1-2 years	34	34.00
3-4years	12	12.00
5-6 years	8	8.00
Breed		
Caucasian	30	30.00
German shepherd	24	24.00
Rottweiler	2	2.00
Mastiff	4	4.00
Local	40	40.00
Sex		
Female	46	46.00
Male	54	54.00
Management system		
Extensive	14	14.00
Semi- Intensive	24	24.00
Intensive	62	62.00

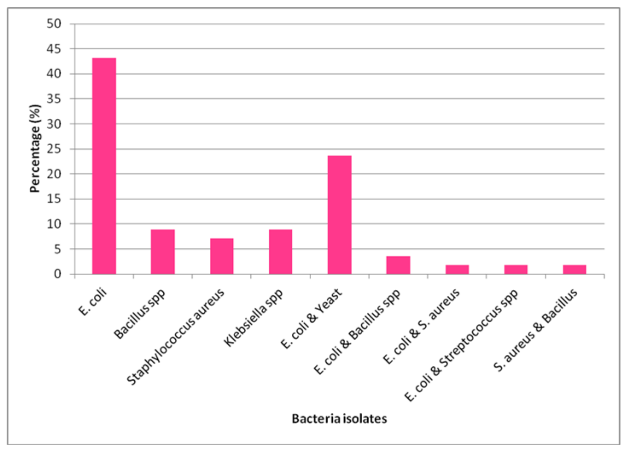

Fig. 2: Bacteria colonizing oral cavities of dogs

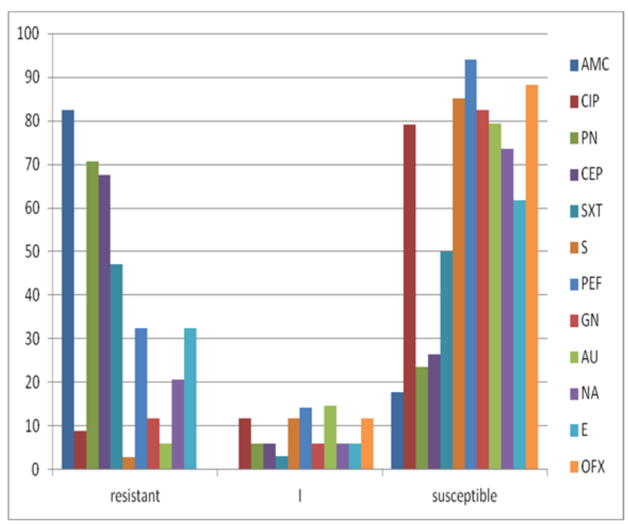

Fig 3: Susceptibility pattern of *Escherichia coli* isolates from oral

cavity of dogs

Key: Tarivid (OFX), Ceporex (CEP), Gentamycin (GN), Augumentin (AU), Nalidixic acid (NA), Ciprofloxacin (CIP), Streptomycin (S), Peflacin (PEF), Septrin (SXT), Ampicilin (PN), Erythromycin (E), Amoxicillin (AMC)

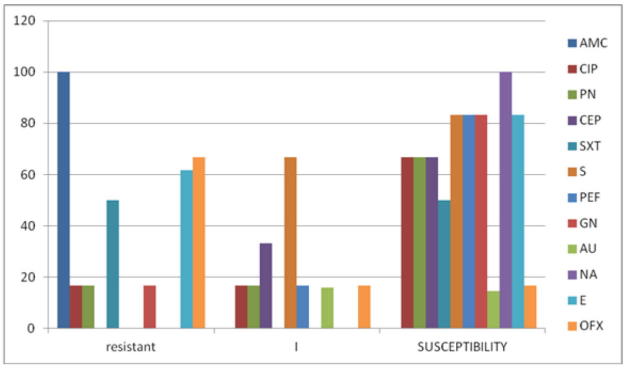

Fig 4: Susceptibility Pattern of *Klebsiella* Isolates from Oral Cavity of Dog**.**

Key: Tarivid (OFX), Ceporex (CEP), Gentamycin (GN), Augumentin (AU), Nalidixic acid (NA), Ciprofloxacin (CIP), Streptomycin (S), Peflacin (PEF), Septrin (SXT), Ampicilin (PN), Erythromycin (E), Amoxicillin (AMC)

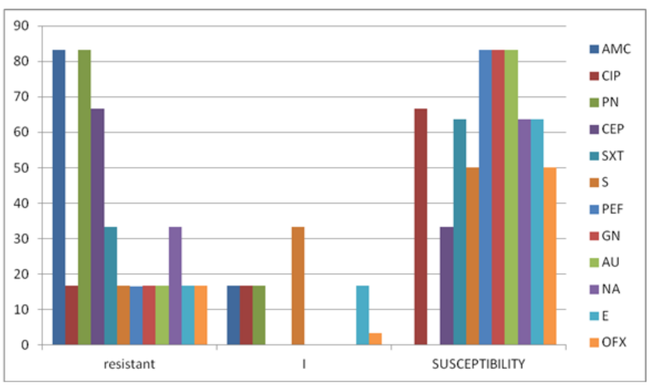

Fig 5: Susceptibility pattern of *Bacillus* Isolates from oral cavity of dogs.

Key: Tarivid (OFX), Ceporex (CEP), Gentamycin (GN), Augumentin (AU), Nalidixic acid (NA), Ciprofloxacin (CIP), Streptomycin (S), Peflacin (PEF), Septrin (SXT), Ampicilin (PN), Erythromycin (E), Amoxicillin (AMC)

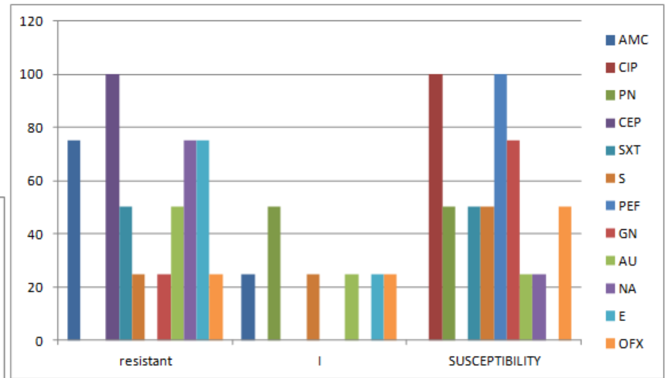

Fig 6: Susceptibility patterns of *Staphylococcus aureaus* isolates from oral cavities of dogs

Key: Tarivid (OFX), Ceporex (CEP), Gentamycin (GN), Augumentin (AU), Nalidixic acid (NA), Ciprofloxacin (CIP), Streptomycin (S), Peflacin (PEF), Septrin (SXT), Ampicilin (PN), Erythromycin (E), Amoxicillin (AMC).

DISCUSSION

Bacteria in dogs' oral cavity may serve as a potential cause of contamination of bite wounds or can be transmitted to humans through licks (Abrahamian and Goldstein, 2011; Oh *et al*., 2015; Ghasemzadeh and Namazi, 2015). Previous studies have reported isolation of multidrug-resistant bacteria of public health importance in people and companion animals (Hemsworth and Pizer, 2006). In this study, we isolated diverse drug resistant bacteria species from the oral cavities of apparently healthy dogs in Jos, Plateau State Nigeria. Isolation of the bacteria was across different breeds, ages, sexes and management systems. Osinubi *et al***.** (2003) and Ofukwu *et al***. (**2008) reported the isolation of aerobic bacteria such as *E. coli, Streptococcus, Staphylococcus, Klebsiella* and *Bacillus* and is consistent with our findings. We did not isolate aerobic bacteria such as *Corynebacterium, Listeria, Morixiella* and *Proteus* as reported by Osinubi *et al*. (2003) and Ofukwu *et al*., (2008). Consequently, the study showed that there is no breed, age, and sex or management system variation in the colonization or distribution of bacteria in the oral cavities of apparently healthy dogs. The study invariably suggests that all breeds of dogs irrespective of management type may be exposed to the bacterial species from the environment or contaminated foods and may serve as potential source of transmission to humans.

Although the bacteria species isolated in this study are not naturally pathogenic they may become pathogenic in situations such as immune suppression. Infections with some of the organisms may lead to pneumonia, urinary tract infections, meningitis, osteomyelitis, wound infections, surgical site infection and endocarditis (Neu, 1992; Elisha *et al*., 2017). Control, prevention and treatment of most infectious diseases have recently become difficult due to the spread of multidrug resistant bacteria. This study has confirmed and revealed a moderate level of antimicrobial resistance among bacterial isolates from the oral cavity of dogs.

Some species of *Staphylococcus* and *Streptococcus* that have been reported to be involved in the pathogenesis of some respiratory and skin infections, along with some members of the Enterobacteriaceae causing gastrointestinal, urogenital diseases and wound contamination are resistant to virtually all of the older antibiotics (Neu, 1992). Clinical isolates of *Staphylococcus aureus*, which is the leading cause of nosocomial infections, have been shown to be increasingly resistant to an array of antimicrobial agents like penicillin, gentamicin, tobramycin, amikacin, ciprofloxacin, clindamycin, erythromycin, chloramphenicol, trimethoprim-sulfamethoxazole and vancomycin (Lowy, 2003).

Most of the isolates in this study were resistant to two or more antibiotics. Apart from the β-lactamase–producing strains, the genera of organisms such as *Staphylococcus*, *Streptococcus*, *Escherichia*, *Klebsiella*, *Shigella*, *Salmonella*, *Proteus*, and *Pasteurella* are susceptible to the semisynthetic broad-spectrum penicillins (ampicillin and amoxicillin) (Scarth, 2006). The resistance of *S. aureus*, *Klebsiella* and *E. coli* to B-lactam antibiotics - amoxicillin and ampicillin in this study may be attributed to the likely production of beta-lactamase enzyme by the organisms. This therefore suggests a possible risk of resistance transfer from dogs due to their close association with humans (Pomba *et al*., 2017). Some of the isolates were susceptible to the quinolones such as ciprofloxacin, nalidixic acid and septrin. Others showed susceptibility to the aminoglygosides-streptomycin (Trimethoprim-sulfamethoxazole) and peflacin. These antibiotics are associated with a greater probability of therapeutic success and could therefore be useful in the management of dog bite wounds or infections associated with the bacteria isolated from the oral cavities of dogs in the area.

Conclusion

This study has revealed different bacteria isolates from the oral cavity of dogs with *E. coli* having the highest proportion. More so most of the isolates displayed resistance to two or more antibiotics. This suggests that dogs' oral cavities are colonized by multi-drug resistant bacteria with possible transmission to humans through licks or contamination of bite wounds. We therefore recommend good dog ownership. Dogs should be managed intensively since only those managed extensively have been proven culpable for transmitting infection through by bite. Inappropriate and indiscriminate use of drugs should be discouraged. Although prophylactic antibiotic treatment has been recommended in high risk wounds, however, such may not be without challenge and may lead to grave outcome since bacterial isolation and antibacterial sensitivity testing is an uncommon practice. Isolation and antibiotic sensitivity of bacteria at bite wound sites should there be carried out before treatment.

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