RESEARCH ARTICLE

Sokoto Journal of Veterinary Sciences
(P-ISSN 1595-093X/ E-ISSN 2315-6201)
Awoyomi & Ojo /*Sokoto Journal of Veterinary Sciences* (2014) 12(3): 47-52
http://dx.doi.org/10.4314/sokjvs.v12i3.9

Antimicrobial resistance in aerobic bacteria isolated from oral cavities of hunting dogs in rural areas of Ogun State, Nigeria

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Abstract

Aerobic bacterial organisms in oral cavities of hunting dogs could infect bite wounds. Oral swabs from hunting dogs in rural communities located in a south western state of Nigeria were collected and investigated for aerobic bacteria. Sixty two samples examined yielded a total yield of 101 aerobic bacterial isolates belonging to 12 genera. The species of bacteria detected included *Bacillus* spp*, Pseudomonas* spp*, Staphylococcus* spp*, Streptococcus* spp*, Aeromonas* spp*, Burkholderia* spp*, Citrobacter* spp*, Escherichia* spp*, Enterobacter* spp*, Pasteurella* spp*, Burkholderia* spp*, Shewanella* spp and *Vibrio* spp*.* Susceptibility of all identified isolates to antimicrobial agents was determined by the standard Kirby-Bauer disk diffusion method. In all, the isolates showed resistance to ampicillin (90.1%), chloramphenicol (79.2%), ciprofloxacin (33.7%), enrofloxacin (42.6%), gentamicin (74.4%), nalidixic acid (82.2%), neomycin (80.2%), norfloxacin (42.6%), penicillin (75.2%), sulphamethoxazole (91.1%), streptomycin (88.1%), tetracycline (90.1%), amoxicillin/clavulanic acid (55.4%). This study reinforces the need for dog bite wound microbial culture and antimicrobial sensitivity test as isolates showed varied antimicrobial susceptibility patterns. The oral cavities of hunting dogs are laden with multi-drug resistant bacteria of significant public health importance that could be transferred to humans through contaminated hunted games and bite wound.

Keywords: Aerobic bacteria, Antimicrobial resistance, Dogs, Oral cavity, Public health

Received: 05-01-2014 Accepted: 12-11-2014

Introduction

Humans keep dogs for different reasons as influenced by culture, status, social interests, religious convictions, and economic activities (Wandeler *et al*., 1993). Humans have benefitted a great deal from dog keeping and many consider dogs as loyal friends (McNicholas *et al.*, 2005). Dog keeping is relevant to all categories of people irrespective of socioeconomic background and geographical location (Westgarth *et al.*, 2007). In the rural areas, dogs are also used for hunting game animals which are sold to provide additional income for household. Hunted games are also consumed by hunters and their family members as a source of protein (El-Yuguda *et al.,* 2007). Dogs are organised into hunting packs with frequent hunting expedition. Rural dogs which lack veterinary care and are usually unvaccinated maintain very close contact with humans and are left to roam about without restriction. These ferociously looking hunting dogs are common sights on streets in peri-urban and rural areas and may attack passers-by inflicting serious wound requiring medical attention.

Dog bite constitutes a significant hazard associated with dog keeping (Abrahamian & Goldstein, 2011). Members of dog-keeping household and strangers are exposed to this hazard. Bite wound is an important entry of bacterial contaminants leading to wound infections (Goldstein, 1992). In addition, bite sites may serve as route of transmission of other pathogens associated with systemic diseases such as rabies. Most often, the types of bacteria found in bite wound infection are similar to those present in the oral cavity of the biting dogs (Abrahamian & Goldstein, 2011). Therefore, knowledge of the microbial flora of the oral cavity of dogs will be useful in predicting the likely bacteria to be found in infected bite wounds inflicted by dogs. Mixed bacterial infections are common in bite wound infections (Talan *et al.,* 1999).

Thus, the present study investigated the aerobic bacterial microflora of the oral cavity of nomadic hunting dogs in Abeokuta and determined susceptibility of the bacterial isolates to antimicrobial agents including those commonly used for the topical and systemic treatment of wound infections in humans.

Materials and methods

Sample collection

Oral swabs were collected from 62 hunting dogs in rural areas of Ogun State, South Western Nigeria. Sample collection was carried out for four weeks, between July and August, 2011. Twelve rural communities from four local government areas were included in this study. One local government was selected from each of the four geo-political zones of Ogun State while three communities were included from each of the selected local government. Minimum of five dogs were sampled from each of the twelve communities. Samples were collected using sterile swab sticks, labelled and preserved in icepacks for immediate transportation to the laboratory. The dogs sampled consisted of both males and females of ages ranging from six months and above. Furthermore, they were all indigenous in origin. The dogs were apparently healthy and previously received no antimicrobial therapy. The dogs were mainly scavengers but may sometimes receive household leftover food.

Isolation and identification of bacteria

Each sample was subjected to non-selective 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. Biochemical tests included oxidase, catalase and substrate utilization as determined by commercial biochemical test kits. Gram-negative bacteria were identified using biochemical tests kits (Microbact GNB 24E®, Oxoid, Basingstoke, UK). Isolates suspected to be *Staphylococcus* specie as revealed by microscopy were tested for coagulase production using fresh rabbit plasma and biochemical properties (Microbact Staph 12S, Oxoid, Basingstoke, UK). Results of biochemical tests were interpreted with computer software (Oxoid Microbact® 2000 version 2.03) for the identification of all tested isolates. The cut-off percentage of the Microbact 24E was 75% and above.

Antimicrobial susceptibility test

Selection of the antibiotics panel was based on their spectrum of activity and frequency of use by clinicians in treatment of bite wounds. The susceptibility of all identified isolates to antimicrobial agents (Table 1) was determined by the standard Kirby-Bauer disk diffusion method. A single colony of the isolate under test was inoculated into TSB and incubated for 8 to 12 hours. After incubation, the turbidity of the TSB culture was adjusted to 0.5 McFarland standards. A sterile swab was dipped into the adjusted TSB culture and inoculated onto Mueller-Hinton agar (MHA) (Oxoid, Basingstoke, UK) plate by swabbing the entire surface of the MHA. The antimicrobial disks were each placed firmed on the inoculated MHA plate. Concentrations of antibiotic disks used were : Ampicillin-10 µg; Amoxicillin/clavulanic acid-30 µg; Chloramphenicol 5µg; Ciprofloxacin 5µg; Enrofloxacin 5µg; Gentamicin 10µg; Nalidixic acid 30µg; Neomycin 30µg; Norfloxacin 10µg; Penicillin 10units; Sulphamethoxazole 23.75/1.25 µg; streptomycin 10µg; Tetracycline 30µg and Methicillin 5µg. The plates were incubated at 37 ^{o}C for 18-24 hours. After incubation, the diameter of the clear zone of inhibition around each antimicrobial disk was measured (in millimetre) and the result interpreted in accordance with the recommendation of Clinical and Laboratory Standards Institute (CLSI, 2008). *Escherichia coli* American Type Culture Collection (ATCC) 25922 was included for quality control.

Results

Oral swab from sixty two dogs were collected with cultures yielding a total of 101 aerobic bacterial isolates. Nineteen (19) different species of bacteria were identified. The most commonly encountered bacteria in the oral cavities of hunting dogs were Bacillus spp 41(40.6%). Others are Staphylococcus spp 19(18.8%), Pseudomonas spp 8(7.9%), Burkholderia spp 8(7.9%), Streptococcus spp 7(6.9%), Escherichia spp 5(5%), Aeromonas spp 3(3%), Shewanella spp 3(3%), Citrobacter spp 2(1.9%), Pasteurella spp 2(1.9%),Vibrio spp 2(1.9%) and Enterobacter spp 1(1%) (Tables 1-3).

Table 1: Antimicrobials susceptibility patterns of gram negative miscellaneous bacteria isolated from oral cavities of hunting dogs in Ogun State

Bacterial	Frequency	Antimicrobials resistance of isolates (%)												
Isolate	of isolation	AMP	AMC	CHL	CIP	ENR	GEN	NAL	NEO	NOR	PEN	SUL	STR	TET
Aeromonas hydrophilia	3 (3.0)	3 (100)	1 (33.3)	2 (66.6)	1 (33.3)	1 (33.3)	1 (33.3)	3 (100)	2 (66.6)	2 (66.6)	3 (100)	2 (66.6)	1 (33.3)	3 (100)
Burkholderia cepacia	3 (3.0)	3 (100)	3 (100)	2 (66.6)	2 (66.6)	2 (66.6)	3 (100)	3 (100)	3 (100)	2 (6.6)	3 (100)	3 (100)	3 (100)	3 (100)
Burkholderia pseudomallei	5 (5 .0)	5 (100)	5 (100)	4 (80)	(80)	5 (100)	5 (100)	5 (100)	5 (100)	4 (80)	5 (100)	5 (100)	5 (100)	5(100)
Pasteurella multocida	2 (2.0)	2 (100)	1 (50)	1 (50)	0 (0)	0 (0)	2 (100)	1 (50)	2 (100)	0 (0)	1 (50)	2 (100)	1 (50)	1 (50)
Pseudomonas aeruginosa	6 (5.9)	6 (100)	6 (100)	6 (100)	4 (66.7)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	5 (83.3)	5 (83.3
Pseudomonas fluorescens	1 (1.0)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
Pseudomonas spp	1(1.0)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
Shewanella putrefaciens	3 (3.0)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)
Vibrio alginolyticus	2 (2.0)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)	1 (50)	0 (0)	0 (0)

Key: AMP: Ampicillin; AMC: Amoxicillin/clavulanic acid; CHL: Chloramphenicol; CIP: Ciprofloxacin; ENR: Enrofloxacin; GEN: Gentamici NAL: Nalidixic acid; NEO: Neomycin; NOR: Norfloxacin; PEN: Penicillin; SUL: Sulphamethoxazole; STR: streptomycin; TET: Tetracycline;

Table 2: Antimicrobials susceptibility patterns of enterobactericeae isolated from oral cavities of hunting dogs in Ogun State

Bacterial Isolate	Frequency of isolation	Antimicrobials resistance of isolates (%)												
		AMP	AMC	CHL	CIP	ENR	GEN	NAL	NEO	NOR	PEN	SUL	STR	TET
Citrobacter youngae	2 (2.0)	2 (50)	0 (0)	1 (50)	1 (50)	1 (50)	1 (50)	2 (100)	1 (50)	1 (50)	2 (100)	0 (0)	0 (0)	0 (0)
E. coli1	4 (4.0)	4 (100)	2 (50)	3 (75)	1 (25)	1 (25)	1 (25)	4 (100)	1 (25)	1 (25)	4 (100)	3 (75)	3 (75)	3 (75)
Enterobacter cloacae	1 (1.0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)
Escherichia amnigenus biogrp 1	1 (1.0)	1 (100)	1 (100)	1 (100)	0 (0)	0 (100)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)

KEY: AMP: Ampicillin; AMC: Amoxicillin/Clavulanic acid; CHL: Chloramphenicol; CIP: Ciprofloxacin; ENR: Enrofloxacin; GEN: Gentamici NAL: Nalidixic acid; NEO: Neomycin; NOR: Norfloxacin; PEN: Penicillin; SUL: Sulphamethoxazole; STR: streptomycin; TET: Tetracycline

Table 3: Antimicrobials susceptibility patterns of gram positive bacteria isolated from oral cavities of hunting dogs in Ogun State

Bacterial Isolate	Frequency of isolation	Antimicrobials resistance of isolates (%)													
		AMP	AMC	CHL	CIP	ENR	GEN	NAL	NEO	NOR	PEN	SUL	STR	TET	MET
Bacillus spp	41 (40.6)	34 (82.9)	15(6.6)	29(71.7)	10 (24.4)	12(29.3)	19 (6.3)	32 (78.1)	32 (78.1)	12 (29.3)	23 (56.1)	38(92.7)	38 (92.7)	39 (95.1)	NT
Staphylococcus aureus	7 (6.9)	6 (85.7)	6 (85.7)	7 (100)	3 (42.9)	3 (42.9)	6 (85.7)	6 (85.7)	6 (85.7)	5 (71.4)	5 (71.4)	7 (100)	7 (100)	7 (100)	5 (71.4)
Staphylococcus intermedius	6 (5.9)	6 (100)	1 (16.7)	6 (100)	1 (16.7)	3 (50)	6 (100)	5 (83.3)	5 (83.3)	2 (33.4)	4 (66.7)	6 (100)	6 (100)	6 (100)	1 (16.7)
Streptococcus pyogenes	1 (1.0)	1 (100)	1 (100)	1 (100)	0 (100)	0 (100)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	NT
Streptococcus spp	6 (5.9)	6 (100)	4 (66.7)	6 (100)	2 (33.4)	3 (50)	6 (100)	6 (100)	6 (100)	3 (50)	6 (100)	6 (100)	6 (100)	6 (100)	NT
Coagulase negative Staphylococcus spp	6 (5.9)	6 (100)	5 (83.3)	5 (83.3)	0 (0)	1 (16.7)	2 (33.4)	4 (66.7)	5 (83.3)	0 (0)	6 (100)	6 (100)	6 (100)	6 (100)	2 (33.4)

KEY: AMP: Ampicillin; AMC: Amoxicillin/clavulanic acid; CHL: Chloramphenicol; CIP: Ciprofloxacin; ENR: Enrofloxacin; GEN: Gentamici NAL: Nalidixic acid; NEO: Neomycin; NOR: Norfloxacin; PEN: Penicillin; SUL: Sulphamethoxazole; STR: streptomycin; TET: Tetracycline; MET: Methicillin; NT : Not tested

The isolates showed varied degrees of resistance to tested antimicrobials. Overall, there was resistance to ampicillin (90.1%), chloramphenicol (79.2%), ciprofloxacin (33.7%), enrofloxacin (42.6%), gentamicin (74.4%), nalidixic acid (82.2%), neomycin (80.2%), norfloxacin (42.6%), penicillin (75.2%), sulphamethoxazole (91.1%), streptomycin (88.1%), tetracycline (90.1%), amoxicillin/clavulanic acid (55.4%) . Twenty one of the isolates were completely resistant to all the antimicrobials used in the study.

Discussion

The oral cavities of dogs is laden with diverse bacterial species as revealed by this study. A large proportion of dog bite wounds are infected with aerobic bacteria and majority of bacteria isolated from bite wounds are those transferred from the oral cavity of biting animals (Abrahamian & Goldstein, 2011). The aerobic bacteria encountered in the present study are similar to those reported by other authors (Ofukwu *et al.,* 2008; Osinubi *et al.,* 2003). Bacterial species such as *E. coli, Streptococcus, Staphylococcus, Pasteurella* and *Bacillus* were commonly reported as part of the aerobic microflora of the oral cavity of dogs similar to findings in the present study. However, the frequency of isolation of these bacteria as observed in the present study differs from those reported by earlier workers. For instance, *E. coli* (51.6%) was the most predominant bacterial species reported by Ofukwu *et al.* (2008) but *Bacillus* specie predominated in the present study. Previous studies also reported the isolation of some aerobic bacteria species such as *Corynebacterium, Listeria, Moraxella*, *Proteus* and *Klebsiella* which were absent in the present study (Ofukwu *et al.,* 2008; Osinubi *et al.,* 2003). Conversely, bacterial species including *Aeromonas, Vibrio* and *Shewanella* were encountered in the present study but were not reported by the earlier workers. Environmental factors, diet and living conditions may influence the type of bacterial species and their frequency of occurrence in the oral cavity of dogs (Abrahamian & Goldstein, 2011). Some bacteria also occur transiently and are not among the regular microflora of the oral cavity (Abrahamian & Goldstein, 2011). All the dogs examined in the present study lived as scavengers and were used for hunting by their owners. The dogs were therefore exposed to myriads of bacterial species from the environment, contaminated foods and hunted prey.

Dog bite injuries can become infected leading to severe purulent wound, abscess formation, cellulitis and lymphangitis (Abrahamian & Goldstein, 2011). Aerobic bacteria isolated from oral cavity of hunting dogs in the present study should be considered important contributor to dog bite wound infections. Bacterial co-infection is common in bite wound. It has been reported that up to 48% of dog bite wounds are infected with more than one bacterial species (Abrahamian & Goldstein, 2011). Although in most cases both aerobic and anaerobic bacteria are involved, pure aerobic culture reportedly account for up to 42% of cases. Bacterial species isolated from the oral cavity of hunting dogs in the present study have been reported in cases of dog bite wounds (Talan *et al.,* 1999).

Antimicrobial agents are commonly used for the treatment of bite wound infections. The present study revealed a high level of antimicrobial resistance among bacterial isolates from the oral cavity of hunting dogs. This portends real threat to the efficacy of antimicrobial chemotherapy in the treatment of bite wound infections. Of particular interest is the detection of methicillin-resistant *Staphylococcus aureus* and other methicillin-resistant *Staphylococcus* strains which are of significant public health interest. *Staphylococcus intermedius* which is a recognised commensal found on the mucosal surfaces of the oral and nasal cavities as well as on the skin of apparently healthy dogs has also been implicated in canine invasive disease (Talan *et al.,* 1989). Human infections with antimicrobial-resistant *S. intermedius* have been reported (Pottumarthy *et al.,* 2004). Globally, methicillin-resistant *S. aureus* (MRSA) is generating concerns because of the high morbidity and mortality linked with its infections in humans (Boucher & Corey 2008). Dogs can act as reservoirs of MRSA transmissible to humans. Studies have shown that humans and their pets harbour similar MRSA strains (Baptiste *et al.,* 2005). An earlier study implicated dog in cases of MRSA infections in humans (Manian, 2003). Methicillin-resistant *S. aureus* present in the oral cavities of dogs are as shown by the present study could be transferred to bite victims leading to possibly fatal infection.

Conclusively, the present study established the presence of resistant bacteria of diverse species in the oral cavities of hunting dogs in the study area. Apart from the possibility of infecting bite wounds, many of the bacteria can cause opportunistic infections and can be involved in food-borne infections. They could be transferred to humans through dog bite and consumption of contaminated undercooked meat of hunted animals. Close contact and licking can also facilitate dog-to-human transmission of the pathogens from oral cavity of hunting dogs. Factors such as immune-suppression, malnutrition and stress that can aid human susceptibility to opportunistic pathogens are common among the rural poor. Responsible dog ownership, movement restriction of dogs and improvement in personal hygiene can limit the zoonotic transmission of these pathogens from dogs to humans.

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