Bacterial Infections of the Upper Respiratory Tract of Different Breeds of Dog in Abeokuta, Ogun State, Nigeria

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Abstract: Dogs are domestic animals as well as human pets with potential zoonotic respiratory infections. Nasopharyngeal samples were collected from a total of fifty-five (55) dogs at the Veterinary Teaching Hospital (VTH), Federal University of Agriculture, Abeokuta (FUNAAB), Nigeria. Ten (10) breeds were examined for probable bacteria responsible for upper respiratory tract infection (URTI) and antibiotic resistance among the isolates. The isolates per breed with age as a factor, were microbiological screened, while antimicrobial susceptibility test (AST) was performed by Kirby-Bauer's disc diffusion method with their minimum inhibitory concentration (MIC). The URTI was highest (49.0%) for dogs younger than 12 months, while the least percentage of 5.5% was recorded for dogs between ages 6 and 10 years. The decreasing order of URTI rate based on breed was; Alsatian (43.6%)>Boerboel (20.0%)>Italian mastiff>Terrier cross (1.8%). The frequency of occurrence of ten identified bacterial species were *Escherichia coli* (83.1%), *Citrobacter freundii* (73.4%), *Staphylococcus aureus* (67.5%), *Klebsiella oxytoca* (65.2%), *Bacillus subtilis* (57.6%), *Staphylococcus saprophyticus* (40%), *Pseudomonas aeruginosa* (38.2%), *Streptococcus* spp. (18.2%), *Proteus mirabilis* (14.5%) and *Haemophilus* spp. (5.8%). All the isolates expressed significant differences ($P<0.05$) across all the parameters tested and were also 100% resistant to at least one of the antibiotics tested. Percentage susceptibility rate (%) to nitrofurantoin (100), ciprofloxacin and amoxycillin (90.0), ceftriaxone (10.0), while augmentin was completely resisted by all the isolates (0%). The study revealed that most pet-dogs in the sampled area were potential carriers of antibiotic-resistant bacterial strains. More public awareness aimed at curtailing the spread of these pathogenic agents is highly recommended.

Key word: Bacterial infection, breeds of dog, human pets, upper respiratory tract

INTRODUCTION

Genetic evidence shows an evolutionary split between the modern dog's lineage and the modern wolf's lineage around 100,000 years ago, but the oldest fossil specimens genetically linked to the modern dog's lineage dated to approximately 33,000-36,000 years ago (Laura *et al*., 2017; Mietje *et.al*., 2009). Dogs are domestic animals having close contact with humans (Dewey and Bhagat, 2002; Natalie *et al*., 2019) and are becoming more economically important day after day as dogs are being used as pets, security, game hunting and amazingly, as meat in some parts of the country (Druzhkova *et. al*., 2013). Through genetic selective breeding, dogs have been characterized into hundreds of various breeds, and shows more behavioral and morphological variation than any other land mammal (Spady and Ostrander, 2008; Erin *et al*., 2019). The common breeds include; Akita, Boerboel, Bloodhound, Boxer, Bouvier Des Flanders, Bulldog, Bull Mastiff, Chihuahua, Chowchow, Dobermann, German Shepherd, Labrador Retriever, Neapolitan Mastiff, Pit Bull Terrier, Rottweiler, West Highland White Terrier and so on (AKC, 2022).

In Nigeria currently, for security reasons and due to lack of adequate knowledge of implications of zoonosis, there is an increasing population of dog owners, with dogs living freely among people, especially children. This close relationship of pet animals to their owners may constitute potential public health hazard (Ajuwape *et al*., 2006; Paul *et al*., 2020). The upper respiratory tract of dogs includes the nasal cavity, larynx, pharynx and bronchi. These regions harbour many microorganisms due to constants inhalation of potentially contaminated air (Ajuwape *et al*., 2006; Tress *et al*., 2017). Coughing and dyspnea are commonly associated with primary

problems of the respiratory tract and may also occur secondary to disorders of other organs or systems (Buonavoglia and Martella, 2007; Shair *et al*., 2022).

Both young and aged animals are at risk of developing respiratory diseases. At birth, the respiratory systems are incompletely developed; thus, facilitates the introduction and spread of pathogens within the lungs and the alveolar flooding may occur. In aged animals, chronic degenerative changes that disrupt normal mucociliary clearance and immunologic allergy may render the lungs more vulnerable to airborne pathogens and toxic particles (Windsor and Johnson, 2006; Brasier *et al*., 2024).

Commensally known bacteria such as; *Pasteurella multocida*, *Bordetella bronchiseptica*, Streptococci, Staphylococci, Pseudomonads and coliform spp. are autochthonous to the canine nasal passages, nasopharynx and upper trachea, and at least intermittently in the lungs, without causing clinical signs (Windsor *et al.*, 2004; Qekwana *et al*., 2020). Opportunistic infections by these bacteria may occur when defense mechanisms are compromised by infections with primary pathogens (distemper, par influenza virus, or canine type 2 adenovirus in dogs), or diseases such as congestive heart failure and pulmonary neoplasia (Knotek *et al.*, 2001; Yondo *et al*., 2023). Secondary bacterial infections complicate the management of viral respiratory infections of dogs (Maboni *et al*., 2019).

The pathogens may continue to reside in the respiratory tract of convalescent animals. When stressed, these animals may relapse; and can also act as a source of infection for others. Poor management practices (overcrowding) are often associated with poor hygienic and environmental conditions, and the resultant stress increases both the incidence and severity of infections. Conditions that favour the spread of infections often occur in catteries, kennels, pet shops, boarding facilities, and human shelters (Buonavoglia and Martella, 2007).

Poor handling of the dog nasal mucus and sputum have been found to be a major route of spread of bacteria from domestic dog to its handlers (Guardabassi *et al*., 2004). Although, more than 10% of dogs may carry *Staphilococcus aureus* and other pathogenic bacteria which may infect humans (Guardabassi *et al*., 2004). Therefore, there is a need to further prevent increasing resistant bacteria pathogen from infecting human beings. This study aimed at profiling the antimicrobial susceptibility pattern of bacteria associated with the upper respiratory tracts in domesticated dogs in Abeokuta, Ogun State.

MATERIALS AND METHODS

Study area: The study was conducted at the Veterinary Teaching Hospital (VTH), Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Southwest Nigeria, lying within Latitude 70114’07’N and Longitude 3026’15’E (Adenubi *et al*., 2022).

Sample collection and transportation: A total of fifty-five (55) nasopharyngeal samples were aseptically collected from dogs brought to FUNAABVTH for treatment, usually, between the hours of 9:00 am – 11:00 am and immediately transported using sterile swabs from the upper respiratory tracts. The samples were transported in sterile maximum recovery diluent at a maintained at temperature (25^{o}C) in Thermos flask (Master chef vacuum flask MC-F808) to the laboratory for microbiological assessment immediately.

Culturing, characterization, isolation and identification bacterial species: Aliquots of serially diluted nasopharyngeal suspension were inoculated onto sterile nutrient agar plates, blood-enriched agar and MacConkey agar for isolation of aerobic bacteria. The plates were invertedly incubated at 35^{o}C for 48 h. The isolates were identified based on their colonial, morphological and biochemical characteristics according to standard methods (Barrow and Feltham, 1993; Cheesbrough, 2006), while the identification was done according to the

description of Bergey's manual of determinative bacteriology (Bergy, 2000).

Antimicrobial susceptibility screening of the bacterial isolates: Different classes of antibiotics were tested against the isolates by the Kirby-Bauer disc diffusion method on Mueller-Hinton agar according to the method of Hudzicki (2009), using; augmentin (20 µg), ceftriaxone (30 µg), nitrofurantoin (30 µg), gentamicin (10 µg), cotrimoxazole (30 µg), ofloxacin (10 µg), ciprofloxacin (10 µg), amoxicillin (10 µg), pefloxacin (10 µg), and tetracycline (30 µg) respectively. One hundred microliter of 0.5 MacFarland standardized broth culture (average of 1.6 x 10^7) of each isolate was spread on Mueller-Hinton agar and allowed to air-dry aseptically. Each antibiotic disc was carefully placed at about 20 mm distance from each other on the inoculated agar and incubated invertedly at 35^{o}C for 24 h. The zones of inhibition were measured and interpreted as sensitive (S), intermediate (I) and resistant (R), according to the interpretation chart of CLSI (2018).

Minimum inhibitory concentration (MIC) of bacterial isolates: Standard broth micro-dilution method was used to determine the MIC of the isolates. All the isolates were tested against the following antibiotics dilution ranges in micro tubes; cotrimoxazole (0.5-64µg/ml), augmentin (0.5-32µg/ml), gentamycin (0.5-64 µg/ml), ciprofloxacin (0.5-64 µg/ml), ceftriaxone (1-64 µg/ml), pefloxacin (0.25-128 µg/ml), ofloxacin (0.25-128 µg/ml), tetracycline (0.25-64 µg/ml), nitrofurantoin (0.25-64 µg/ml) and amoxycillin (0.25-64 µg/ml). Each antibiotic was serially diluted in 1% peptone of 100 µl according to their respective ranges and equal volume of 100 µl of overnight 0.5 MacFarland standardized broth culture was added to all the dilution ranges from well 1 to well 10 and incubated at 35^{o}C for 24 h. Turbid wells were indicated to have growth, while clear wells were identified to have no growth after incubation. The MIC of each antibiotic to the resistant organism was noted as the highest dilution showing no growth. The respective MIC of each isolate was determined and interpreted according to CLSI (2018) recommended guidelines.

Statistical analysis of the obtained data sets: Descriptive statistics was used to present the rate of the isolation of the bacteria using percentages, average and bar chart, while Chi-square was used to determine the significant of the bacteria isolates obtained from the dogs examined at 95% confidence interval of p-value less than 0.05 using SPSS version 16 of 2003.

RESULTS AND DISCUSSION

Majority of the dogs examined were young puppies which are often carried around, played with and used for sporting activities. Percentage occurrence of URTI was highest (43.0) in Alsatian and least (2.0) in Italian mastiff, Mongrel, Caucasian, Neapolitan mastiff and Terrier cross respectively (Figure 1). These severe infections such as; pneumonia or bronchopneumonia occur when dogs come in contact with pathogenic bacterial agents (Timoney, 2004). Table 1 shows both cultural and morphological characteristics of 10 bacterial isolates obtained from the nasal swab of the dogs examined, while biochemical responses of each isolate are presented in Table 2 respectively.

Escherichia coli recorded highest (83.1%) while *Haemophilus sp.* had the least occurrence (5.8%) as shown in Figure 2. The highest percentage occurrence recorded for *E. coli* and *C. freundii* could have resulted from faecal transmission of pathogens through unhygienic handling of animal discharges. Also, unrestrained dogs occasionally scavenge food crumbs in refuse and dirty areas thereby contracting *E. coli* which could easily be transmitted. *Staphylococcus aureus* is an important cause of human nosocomial and community-acquired infections globally and it is usually found responsible for numerous respiratory infections in dogs (Gortel *et al.,* 1999). The transmission of *S. aureus* between pet dogs and their owners was reported by Manian, (2003). A lot of health risks are posed by

contracting *S. aureus* infection from dog through nasal discharge such as sputum, mucus, blood and saliva (Pesavento *et al.*, 2008).

As shown in Table 3, all the isolates were resistant to at least one of the antibiotics tested. Complete susceptibility to nitrofurantoin (100%), ciprofloxacin and amoxycillin (90.0%) were recorded, while 0.0% and 10.0% susceptibility rate were recorded for augmentin and ceftriaxone respectively. The antibiotic susceptibility pattern of the nasal bacterial isolates depicted increasing rate of antibiotic resistance among the bacteria resident in dogs. In the last few years, methicillin-resistant *Staphylococcus aureus* (MRSA) has gained world-wide attention as a human pathogen in hospitals and in communities (Bourély *et al.*, 2019). Recent reports confirmed MRSA infection and colonization in dogs and cats (Walther *et al.*, 2008), thereby, indicating that the resistant strain is becoming a pathogen of animals as well as human involving wounds and post-operative infections (Weese *et al*., 2006).

Streptococcus species which is found as commensally organism found on the tonsils, URT, skin and urinogenital tract of dogs is now considered an opportunistic pathogen (Gibson and Richardson, 2008).

Table 4 shows minimum inhibitory concentration (MIC) of antibiotic agents against the bacterial isolates. The MIC ≥16 µg/ml is interpreted as resistant according to CLSI (2018) guideline and resistance rate of 100% was recorded to augmentin, 90% resistant to ceftriaxone and 60% to gentamycin. No resistant was shown to nitrofurantoin (0.0%). This may suggest a dangerous trend for public health as many bacterial isolates from dog which infect humans may be very difficult to treat as a result of their drug resistance.

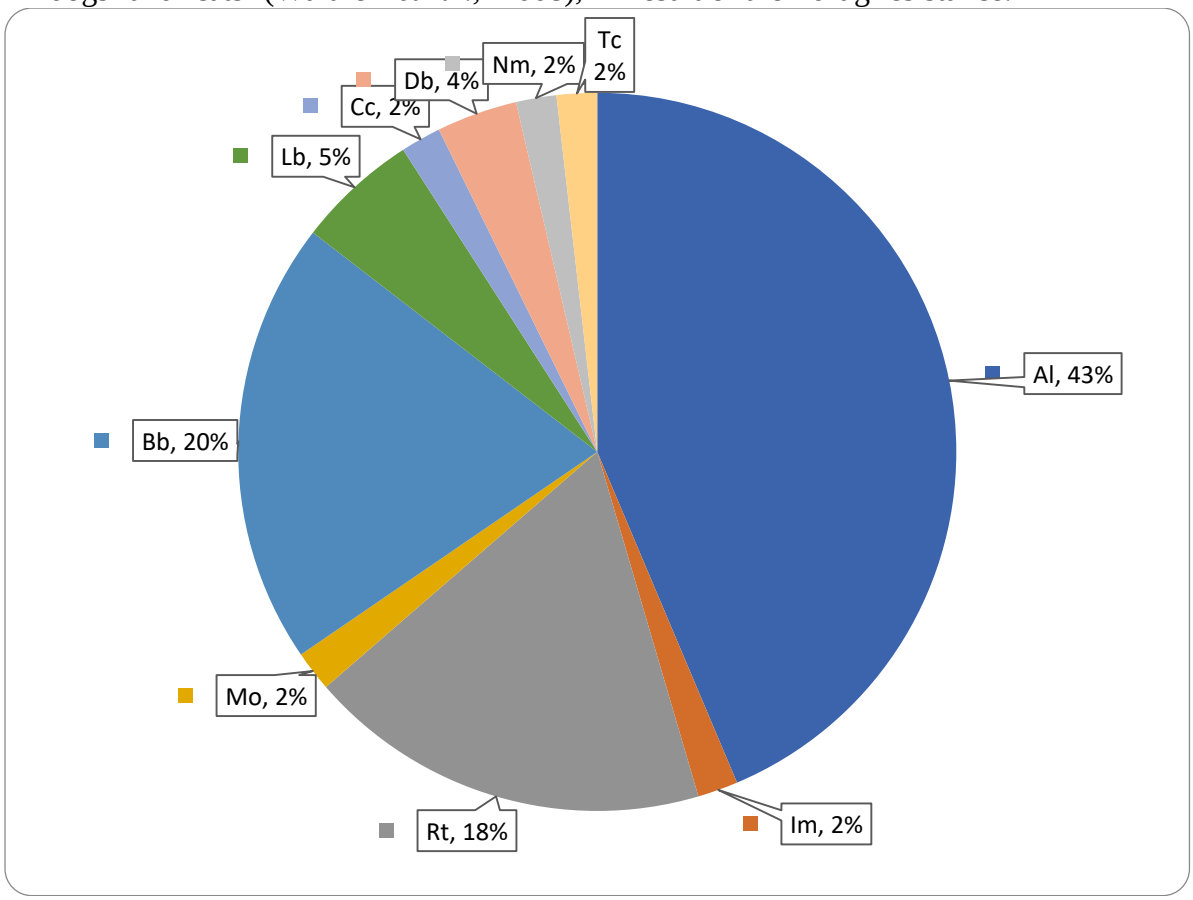

Figure 1: Percentage occurrence per breed of URTI
Al: Alsatian, Im: Italian mastiff, Rt: Rottweiler, Mo: Mongrel, Bb: Boerboel, Lb: Labrador, Cc: Caucasian, Db: Doberman, Nm: Neapolitan mastiff, Tc: Terrier cross

Table 1: Cultural and morphological characteristics of the bacterial species isolated from Upper Respiratory Tracts of Dogs

Isolate code	Cultural characteristics	Morphology
B1	Raised, wet, non-haemolytic, round	GNB
B2	Small, Non-Haemolytic, Round	GNB
B3	Small, translucent, partial haemolysis	GNC
B4	Small, non-lactose fermenter, round	GNB
B5	Yellow, round, non-haemolytic	GPC
B6	Very small, translucent, haemolytic	GPC
B7	Golden yellow, small, haemolytic	GPC
B8	Large, mucoid, wet	GNB
B9	Big, large, wet	GPB
B10	Small, rough, non-lactose fermenters	GNB

Key: GNB = Gram negative bacilli, GPC = Gram positive Cocci

Table 2: Biochemical characteristics of the bacterial isolates from Upper Respiratory Tracts of Dogs

S/N	Code	Gram	Motility	Glucose	Lactose	Mannitol	Maltose	Indole	Methyl Red	Voges Proskauer	Citrate	H_2S	Sucrose	Urea	Oxidase	Coagulase	Catalase	Probable Isolate
1	B1	-	+	+	+	+	+	+	+	-	-	-	NA*	-	-	NA*	+	*Escherichia coli*
2	B2	-	+	+	+	+	+	-	+	-	+	+	-	-	-	-	+	*Citrobacter freundii*
3	B3	-	+	+	+	+	+	+	+	-	-	-	NA*	-	-	NA*	+	*Haemophilus* species
4	B4	-	+	+	-	+	+	-	+	+	-	+	+	+	+	NA*	+	*Pseudomonas aeruginosa*
5	B5	+	-	+	+	+	+	NA*	+	-	+	-	+	+	-	-	+	*Staphylococcus saprophyticus*
6	B6	+	-	+	+	+	+	NA*	+	-	+	-	+	+	-	-	-	*Streptococcus* sp.
7	B7	+	-	+		+	+	NA*	+	-	+	-	+	+	-	+	+	*Staphylococcus aureus*
8	B8	-	-	+	+	+	+	-	-	-	+	-	+	-	-	-	+	*Klebsiella oxytoca*
9	B9	+	+	+	+	+	+	NA*	-	+	NA*	NA*	+	-	-	NA*	+	*Bacillus subtilis*
10	B10	-	+	+	-	-	-	-	+	-	+	+	+	+	-	NA*	+	*Proteus mirabilis*

Key: + = positive reaction, - = negative reaction, NA = not analyzed

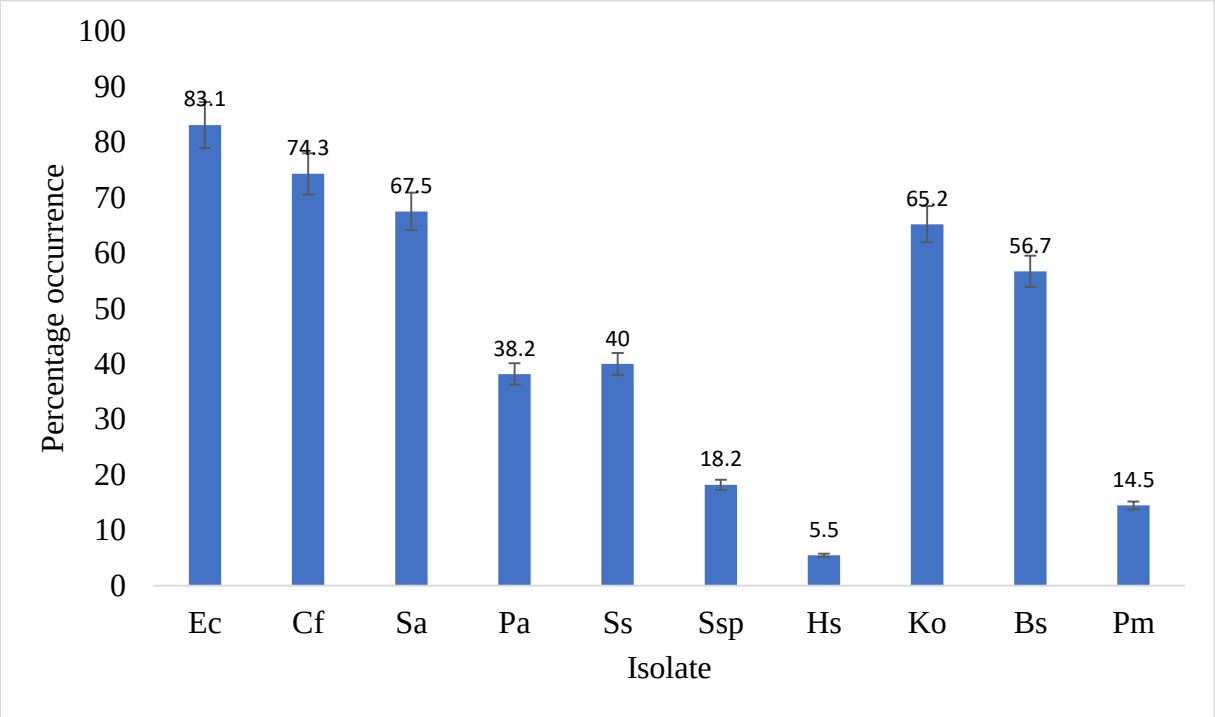

Figure 2: Percentage occurrence of bacterial species isolated from URT of selected dogs
Keys: Ec = *Escherichia coli,* Cf = *Citrobacter freundii,* Sa = *Staphylococcus aureus,* Pa = *Pseudomonas aeruginosa,* Ss = *Staphylococcus saprophyticus,* Ssp = *Streptococcus sp.,* Hs = *Haemophilus species,* Ko = *Klebsiella oxytoca,* Bs = *Bacillus subtilis,* Pm = *Proteus mirabilis.*

Table 3: Antibiotics susceptibly pattern of the bacterial isolates from Upper Respiratory Tracts of Dogs

Isolates	Augmentin	Ceftriaxone	Nitrofurantoin	Gentamycin	Cotrimoxazole	Ofloxacin	Amoxycillin	Ciprofloxacin	Tetracycline	Pefloxacin
Escherichia coli	R	R	S	R	R	R	S	S	R	R
Citrobacter freundii	R	R	S	R	R	R	R	R	S	R
Staphylococcus aureus	R	R	S	R	S	S	S	S	R	R
P.aeruginosa	R	R	S	R	S	S	S	S	S	R
Stapylococcus saprophyticus	R	R	S	R	S	S	S	S	S	S
Streptococcus spp	R	S	S	S	S	S	S	S	R	R
Haemophilus species	R	R	S	S	S	S	S	S	S	S
Klebsiella oxytoca	R	R	S	S	S	S	S	S	S	R
Bacillus subtilis	R	R	S	R	S	S	S	S	S	R
Proteus mirabilis	R	R	S	S	S	S	S	S	S	R
Total susceptibility rate (%)	0.0	10.0	100.0	40.0	80.0	80.0	90.0	90.0	70.0	80.0

Key: R =Resistant, S=Sensitive. (CLSI, 2018).

Table 4: Minimum inhibitory concentration (MIC) of antibiotics against the bacteria isolated from Upper Respiratory Tracts of Dogs

Isolates	Augmentin	Ceftriaxone	Nitrofurantoin	Gentamycin	Cotrimoxazole	Ofloxacin	Amoxicillin	Ciprofloxacin	Tetracycline	Pefloxacin
	MIC≥16 µg/ml									
Escherichia coli	32	32	16	64	64	16	4	2	32	16
Citrobacter freundii	32	32	2	16	16	32	32	32	2	32
Staphylococcus aureus	64	64	1	64	2	2	4	1	16	2
P. aeruginosa	32	32	2	32	2	1	1	2	1	16
Staphylococcus saprophyticus	32	32	2	16	2	2	2	2	2	2
Streptococcus sp.	32	2	2	1	2	1	2	2	32	32
Haemophilus species	32	32	2	2	1	1	1	2	2	4
Klebsiella oxytoca	32	32	2	2	2	1	2	2	2	32
Bacillus subtilis	32	16	1	16	1	8	8	4	4	32
Proteus mirabilis	32	32	2	8	4	4	8	8	8	16
Total susceptibility rate	0.0	10.0	100.0	40.0	80.0	80.0	90.0	90.0	70.0	80.0

CONCLUSION

Dogs and other pet animals living closely with humans could transmit fatal respiratory infections. Also, abuse of antibiotics on dogs could increase drug resistance in bacteria of medical importance associated with domestic dogs. Public awareness on health risks other than bites from pet dogs, regular vaccination and adequate treatment of infected dogs are highly recommended.

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