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ANTIBIOTIC RESISTANCE PROFILING AND MICROBIOTA OF THE UPPER RESPIRATORY TRACT OF APPARENTLY HEALTHY DOGS IN IBADAN, SOUTH WEST NIGERIA.

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Abstract

Background: Rearing of dogs and other pets has become increasingly popular in modern society. Bacterial flora resides within the nasal and oral cavities of dogs and when chanced, can be pathogenic. Certain similarities between humans and dogs portends dangerous behavioral habits that could lead to zoonotic disease transmission. This study was aimed at isolation, identification and antibiotic profiling of bacteria from nasal swabs of apparently healthy dogs. The zoonotic risk was also considered.
Methodology: A total of 173 nasal swabs were collected from 173 apparently healthy dogs. Structured questionnaires were administered to investigate human behavioral habits.
Results: Two hundred and twenty two (222) bacterial isolates were obtained from the culture with ten (10) potentially pathogenic bacteria in the order of *Escherichia coli* (18.5%), *Proteus species* (17.1%), *Staphylococcus aureus* (14.0%), *Klebsiella species* (9.0%), *Acinetobacter species* (9.0%), coagulase negative *Staphylococcus species* (7.7%), *Pseudomonas species* (6.8%), *Actinobacter species* (6.8%), *Citrobacter species* (5.9%) and *Streptococcus species* (5.4%). Overall, the Gram negative isolates showed resistance to ciprofloxacin (9.3%), sparfloxacin (16.0%),perfloxacin (17.3%), ofloxacin (21.6%), chloramphenicol (34..6%), gentamycin (36.4%), streptomycin (37.%), septrin (49.4%), amoxillin (59.3%), augmentin (62.3%) while the Gram positive bacteria showed resistance to ciprofloxacin (3.3%), perfloxacin (6.7%), erythromycin (13.3%), streptomycin (21.7%), rocephin (28.3%), septrin (28.3%), gentamycin (36.7%), zinnacef (68.3%), ampiclox (81.7%) and amoxillin (85.0%). Multi-drug resistance (MDR) to three or more antimicrobials was observed in some of the isolates. Seventy - seven resistance patterns were observed, 16 in Gram positive and 61 in Gram negative bacteria.
Conclusion: This study revealed MDR to two or more antimicrobials in all the isolates. These can pose antibiotic resistance challenges in situation of primary or secondary canine respiratory infections. Also, this study revealed that 82% of the dog owners/ lovers had less than 50cm face-to-face contact with these dogs while playing with them, thus increasing their chances of acquiring MDR bacteria from apparently healthy dogs.

Key words: Antibiotic resistance, Microbiota, Upper Respiratory Tract, Dog, Ibadan.

Introduction

The relationship between people and dogs is unique. Among domesticated hunting of prey animals, dogs are capable of performing a wide variety of roles for human: sheep herding, sniffing out drugs and explosives, hunting of prey and security; breeding purposes and companionship (Pet). To be precise about when the friendship began, is very difficult but a reasonable guess suggest that it has been going strong for more than 14,000 years (Bradshaw 2012; Udell *et al.*, 2008). Despite the fact that we live so closely with dogs, it is not entirely without any health risk. A high zoonotic risk is involved with the increasing number of people, rich and poor, keeping dogs for various purposes without much knowledge on the zoonoses (Omudu *et al.*, 2010).
Surveys of microbiota of the nasal cavities, tonsils, and pharynx of clinically healthy dogs have found many types of aerobic and facultative anaerobic bacteria (Weese, 2007) most of which are zoonotic and may escape into the air while the dog breaths. Greater numbers of organisms are routinely cultured from the rostral than from the caudal nasal cavity of dogs (Craig, 2011). Craig, 2011 reported that the same bacteria flora may not possibly be found in nasal cavity and pharynx in each animal because of marked individual variations, but the presence of a certain range of microbiota can be predicted (Craig, 2011). However, the antibiotic resistance reports of bacteria isolated from clinically healthy animals is continuously increasing (Coates *et al.*, 2002; Davis *et al.*, 2014; Henning *et al.*, 2001; Manian, 2003). Since these resistance factors are transferable, antibiotic sensitive microbiota of human may acquire these resistance factors (Lee, 2003) after effective zoonotic transmission including direct contact with pets, contact with feces from pets, preparation of raw meat and bones for pet consumption, and the handling of commercial pet treats (Cherry *et al.*, 2004; Health Canada, 2005).

Currently, the behavioral habits of dog owners/lovers may expose them to pathogenic agents through either inhalation, ingestion, skin contact etc. The aerosolized transmission of disease can occur through both “droplet” and “airborne” means. Droplet transmission is defined as the transmission of diseases by expelled particles that are likely to settle into another surface quickly, typically within three feet (90 cm) of the source (Practical Guidelines for Infection Control in Health Care Facilities 2005). Thus, for example, for an infection to be caused by droplet transmission, a susceptible individual must be close enough to the source of the infection for the droplet (containing the infectious microorganism) to make contact with the susceptible individual’s respiratory tract, eyes, mouth, nasal passages, and so forth (Gralton *et al.*, 2011). The increase in antibiotic resistance among many potential pathogenic bacteria poses a great threat to humans who inhale aerosolised bacteria from the dog’s nostril, mouth, or fur etc.

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This study was carried out to determine the potential pathogenic bacteria in the nasal region of the upper respiratory tract of apparently healthy dogs, antibiotic resistance patterns of the isolated bacteria and the level of closeness (distance) between the dog owner/handler's face and the body of the dog.

Materials and Methods

Collection of samples

Nasal samples were collected from the vestibules of 173 dogs by using sterile swabs. These dogs were clinically healthy and were used as guards, pets, and for breeding and hunting purposes in urban and rural areas of Ibadan, Oyo state, Nigeria. None of the dogs had apparent bacterial infections or was receiving antimicrobial therapy at the time of sample collection. In an attempt to sample a representative portion of the population, samples were collected at irregular intervals. Also, close observation and structured questionnaire administration were done involving the human with closest interaction with the dog sampled. This was done to determine the actual closest human face to dog body distance when playing with the dog. Only one sample was collected per animal. Samples collected were transported in a cooler with ice packs for delivery to the Department of Veterinary Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria, for bacteriological analysis.

Isolation and identification of bacterial isolates

Each nasal sample was inoculated unto nine milliliters of sterile tryptic soy broth (TSB) (OXOID, Basingstoke, UK) in universal bottles. The broth cultures were incubated at 37°C for 18 to 24 h. After incubation, a loopful of the TSB culture was inoculated onto MacConkey agar, 7% sheep blood agar, Eosin Methylene Blue (EMB) (LAB M, lancashire, UK). These inoculated media were incubated at 37°C for 18 to 24 h. Colonial morphology and Gram staining of all the isolates on the plates were carried out; all the isolates were selected for oxidase and catalase production. Coagulase test was carried out for all the Gram positive cocci in clusters. Haemolysis was observed and recorded. Other biochemical and sugar utilization tests were performed. Results of biochemical tests were interpreted using Cowan and Steel's manual for the identification of medical bacteria 3rd edition (Barrow *et al.*, 2003).

Antimicrobial Susceptibility Test

The susceptibility of identified bacterial isolates to antimicrobial agents was determined by the standard Kirby-Bauer disk diffusion method. Susceptibility to the following antimicrobials was determined for sixty Gram positive bacterial isolates: amoxicillin (30µg), ampiclox (30µg), ciprofloxacin (10µg), rocephin (25µg), perfloxacin (10µg), zinnacef (30µg), erythromycin (10µg), gentamicin (10µg), septrin (30µg), streptomycin (10µg) while One hundred and sixty- five Gram negative bacterial isolates were tested for susceptibility to the following antibiotics: augmentin (30µg), ofloxacin (5µg), gentamycin (10µg) and ciprofloxacin (5µg), perfloxacin (10µg), streptomycin (10µg), amoxicillin (30µg), chloramphenicol (30µg)), septrin (30µg), sparfloxacin (10µg).

Results

A total of 222 bacterial isolates were obtained from 173 nasal swabs of apparently healthy dogs. The isolates included *Escherichia coli* 41 (18.5%) followed by *Proteus species* 38 (17.1%), *Staphylococcus aureus* 31 (14.0%), *Klebsiella species* 20 (9.0%), *Acinetobacter species* 20 (9.0%), coagulase negative *Staphylococcus species* 17 (7.7%), *Pseudomonas species* 15 (6.8%), *Actinobacter species* 15 (6.8%), *Citrobacter species* 13 (5.9%) and *Streptococcus species* 12 (5.4%) (Table 1, Chart 1). *Staphylococcus aureus* and *Escherichia coli* ranked highest in the isolation frequency for Gram positive and Gram negative bacteria respectively (Table 1).

The overall rate of *Staphylococcus aureus* (n= 31) displayed 26 (84%), 26 (84%), 21 (68%), 11 (35.5%), 8 (25.8%), 7 (22.6%), 5 (16.0%), 5 (16.0%), 2 (6.5%), 0 (0.0%) resistance to Ampiclox, Amoxicillin, Zinnacef, Gentamycin, Septrin, Streptomycin, Rocephin, Erythromycin, Perfloxacin and Ciprofloxacin respectively. *Streptococcus species* (n=12) showed 11 (92.0%), 11 (92.0%), 9 (75.0%), 8 (66.7%), 6 (50.0%), 6 (50.0%), 6 (50.0%), 3 (25.0%), 2 (16.7%) and 2 (16.7%) to Ampiclox, Amoxicillin, Zinnacef, Gentamycin, Rocephin, Septrin, Streptomycin, Erythromycin, Perfloxacin and Ciprofloxacin. Coagulase negative *Staphylococcus species* (n=17) had resistance 13 (77.0%), 14 (82.4%), 11 (65.0%), 6 (35.0%), 3 (17.6%), 3 (17.6%), 0 (0.0%), 0 (0.0%), 0 (0.0%) and 0 (0.0%) to Ampiclox, Amoxicillin, Zinnacef, Rocephin, Gentamycin, Septrin, Streptomycin, Perfloxacin, Ciprofloxacin and Erythromycin respectively (Table 2). Generally, more than 50% of the gram positive isolates (range: 68.3% - 85.0%) were resistance to Ampiclox, Zinnacef and Amoxicillin (Table 3, Chart 2) while one isolate of *Staphylococcus aureus* and two isolates of *Streptococcus species* were resistant to eight out of ten antibiotics used (Table 6).

Escherichia coli (n= 41) displayed 22 (53.7%), 20 (48.8%), 16 (39.0%), 14 (34.1%), 12 (29.3%), 9 (22.0%), 8 (19.5%), 6 (14.6%), 4 (9.8%) and 3 (7.3%) to Amoxacillin, Augmentin, Septrin, Streptomycin, Gentamycin, Chloramphenicol, Ofloxacin, Sparfloxacin, Perfloxacin and Ciprofloxacin respectively. *Proteus species* (n=38) had 29 (76.3%), 29 (76.3%), 29 (76.3%), 20 (52.6%), 18 (47.4%), 17 (44.7%), 12 (31.6%), 7 (18.4%), 7 (18.4%) and 6 (15.8%) resistance to Amoxacillin, Augmentin, Septrin, Chloramphenicol, Streptomycin, Gentamycin, Ofloxacin, Sparfloxacin, Perfloxacin and Ciprofloxacin respectively. *Klebsiella species* showed 7 (35.0%), 7 (35.0%), 6 (30.0%), 6 (30.0%), 5 (25.0%), 5 (25.0%), 5 (25.0%), 4 (20.0%), 3 (15.0%) and 1 (5.0%) resistance to Septrin, Chloramphenicol, Amoxacillin, Augmentin, Gentamycin, Perfloxacin, Ofloxacin, Streptomycin, Sparfloxacin and Ciprofloxacin respectively. *Acinetobacter species* (n=20 had resistance 12 (60.0%), 11 (55%), 10 (50%), 9 (45.0%), 9 (45.0%), 7 (35.0%), 4 (20.0%), 4 (20.0%), 4 (20.0%) and 3 (15.0%) to Amoxacillin, Augmentin, Septrin, Gentamycin, Streptomycin, Chloramphenicol, Sparfloxacin, Perfloxacin, Ofloxacin and Ciprofloxacin respectively. *Pseudomonas species* (n=15) showed 14 (93.3%), 9 (60.0%), 8 (53.3%), 8 (53.3%), 6(40.0%), 5 (33.3%), 2 (13.3%), 0 (0.0%), 0 (0.0%) and 0 (0.0%) resistance to Augmentin, Amoxacillin, Septrin, Gentamycin, Streptomycin, Chloramphenicol, Perfloxacin, Sparfloxacin, Ciprofloxacin and Ofloxacin respectively. *Actinobacter species* (n=15) had 10 (66.7%), 9 (60.0%), 5 (33.3%), 5 (33.3%), 4 (26.7%), 4 (26.7%), 3 (20.0%), 3 (20.0%), 3 (20.0%) and 1 (6.7%) resistance to Augmentin, Amoxacillin, Septrin, Streptomycin, Chloramphenicol,

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Gentamycin, Sparfloxacin, Perfloxacin, Ofloxacin and Ciprofloxacin respectively. *Citrobacter species* (n=13) had resistance 11 (84.6%), 9 (69.2%), 5 (38.5%), 4 (30.8%), 5 (38.5%), 4 (30.8%), 3 (23.1%), 3 (23.1%), 3 (23.1%) and 1 (7.7%) to Augmentin, Amoxacillin, Septrin, Chloramphenicol, Streptomycin, Gentamycin, Sparfloxacin, Perfloxacin, Ofloxacin and Ciprofloxacin respectively (Table 4). Generally, more than 50% of the Gram negative isolates (range: 59.3% - 62.3%) were resistance to Amoxicillin and Augmentin (Table 5, Chart 3). One each of *Escherichia coli*, *Klebsiella species*, *Pseudomonas species*, *Actinobacter species* and *Citrobacter species* isolates, two *Acinetobacter species* isolates and four *Proteus species* isolates were resistance to ten different antibiotics (Table 7).

It was obtained from the questionnaire that 82% (123/150) of human had contacts, at different times, less than 50cm human face to dog body contact especially when playing with the dogs. 60 (40%) human contacts respondents belong to urban areas where dogs are majorly used as pet, security and breeding purpose while 63(42%) of human contacts belong to rural areas where dogs are majorly used for hunting purpose.

Table 1: Distribution of bacteria isolates

Bacteria	Frequency (%)
Gram positive isolates	60 (27.0%)
Staphylococcus aureus	31 (14.0%)
Coagulase negative Staphylococcus species	17 (7.7%)
Streptococcus species	12 (5.4%)
Gram negative isolates	162 (73.0%)
Escherichia coli	41 (18.5%)
Proteus species	38 (17.1%)
Klebsiella species	20 (9.0%)
Acinetobacter species	20 (9.0%)
Pseudomonas species	15 (6.8%)
Actinobacter species	15 (6.8%)
Citrobacter species	13 (5.9%)
Total number of isolates	222 (100%)

Chart 1: Percentage distribution of bacteria isolates

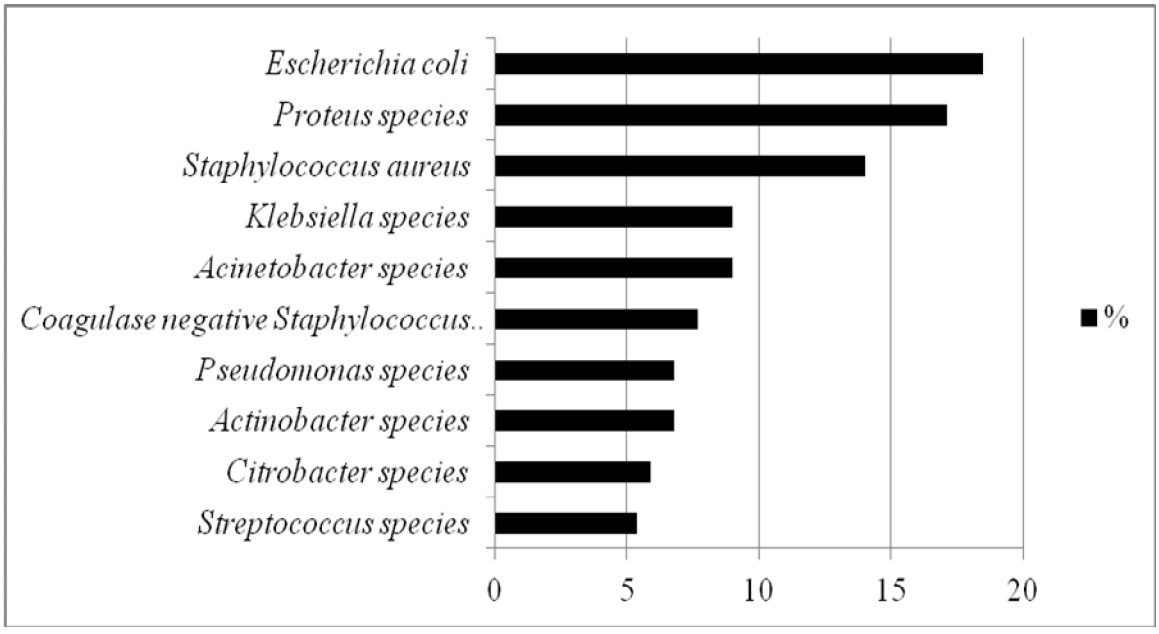

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Table 2: Antibiotic resistance patterns for Gram positive isolates

Antibiotic	Organisms	*Staphylococcus aureus* n= 31	Coagulase negative *Staphylococcus spp.* n= 17	*Streptococcus spp.* n= 12	TOTAL n= 60
Ampiclox	Sensitivity	5(16.0%)	5(23.0%)	1(8.0%)	11(18.3%)
	Resistance	26(84.0%)	12(70.6%)	11(92.0%)	49(81.7%)
Zinnacef	Sensitivity	10(32.0%)	6(35.0%)	3(25.0%)	19(31.7%)
	Resistance	21(68.0%)	11(65.0%)	9(75.0%)	41(68.3%)
Amoxicillin	Sensitivity	5(16.0%)	3(17.6%)	1(8.0%)	9(15.0%)
	Resistance	26(84.0%)	14(82.4%)	11(92.0%)	51(85.0%)
Rocephin	Sensitivity	26(84.0%)	11(65.0%)	6(50.0%)	43(71.7%)
	Resistance	5(16.0%)	6(35.0%)	6(50.0%)	17(28.3%)
Gentamycin	Sensitivity	20(64.5%)	14(82.4%)	4(33.3%)	38(63.3%)
	Resistance	11(35.5%)	3(17.6%)	8(66.7%)	22(36.7%)
Septrin	Sensitivity	23(74.2%)	14(82.4%)	6(50.0%)	43(71.7%)
	Resistance	8(25.8%)	3(17.6%)	6(50.0%)	17(28.3%)
Streptomycin	Sensitivity	24(77.4%)	17(100.0%)	6(50.0%)	47(78.3%)
	Resistance	7(22.6%)	0(0.0%)	6(50.0%)	13(21.7%)
Perfloxacin	Sensitivity	29(93.5%)	17(100.0%)	10(83.3%)	56(93.3%)
	Resistance	2(6.5%)	0(0.0%)	2(16.7%)	4(6.7%)
Ciprofloxacin	Sensitivity	31(100.0%)	17(100.0%)	10(83.3%)	58(96.7%)
	Resistance	0(0.0%)	0(0.0%)	2(16.7%)	2(3.3%)
Erythromycin	Sensitivity	26(84.0%)	17(100.0%)	9(75.0%)	52(86.7%)
	Resistance	5(16.0%)	0(0.0%)	3(25.0%)	8(13.3%)

Table 3: Antibiotic resistance patterns for Gram positive isolates

Test drugs	Amount (µg)	Sensitive	Resistant
Ciprofloxacin	10	58(96.7%)	2(3.3%)
Perfloxacin	10	56(93.3%)	4(6.7%)
Erythromycin	10	52(86.7%)	8(13.3%)
Streptomycin	30	47(78.3%)	13(21.7%)
Rocephin	25	43(71.7%)	17(28.3%)
Septrin	30	43(71.7%)	17(28.3%)
Gentamycin	10	38(63.3%)	22(36.7%)
Zinnacef	20	19(31.7%)	41(68.3%)
Ampiclox	10	11(18.3%)	49(81.7%)
Amoxicillin	30	9(15.0%)	51(85.0%)

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Chart 2: Antibiotic resistance patterns for Gram positive isolates

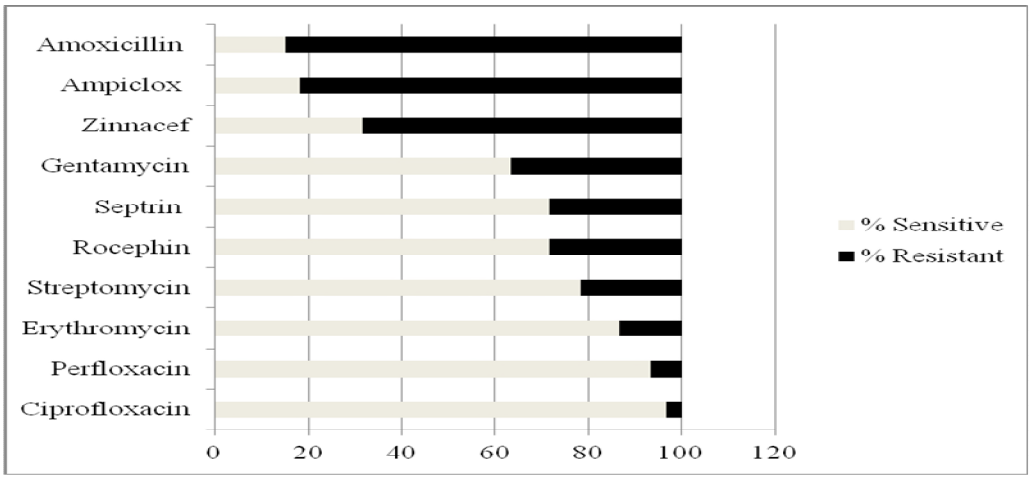

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Table 4: Antibiotic resistance patterns for Gram negative isolates

Antibiotics	Organisms	*Escherichia coli* N= 41	*Proteus species* N=38	*Klebsiella species* N=20	*Acinetobacter species* N=20	*Pseudomonas species* N=15	*Actinobacter species* N=15	*Citrobacter species* N=13	Total N= 162
Amoxacillin	Sensitivity	19 (46.3%)	9 (23.7%)	14 (70.0%)	8 (40.0%)	6 (40.0%)	6 (40.0%)	4 (30.8%)	66 (40.7%)
	Resistance	22 (53.7%)	29 (76.3%)	6 (30.0%)	12 (60.0%)	9 (60.0%)	9 (60.0%)	9 (69.2%)	96 (59.3%)
Augmentin	Sensitivity	21 (51.2%)	9 (23.7%)	14 (70.0%)	9 (45.0%)	1 (6.7%)	5 (33.3%)	2 (15.4%)	61 (37.7%)
	Resistance	20 (48.8%)	29 (76.3%)	6 (30.0%)	11 (55%)	14 (93.3%)	10 (66.7%)	11 (84.6%)	101 (62.3%)
Septrin	Sensitivity	25 (61.0%)	9 (23.7%)	13 (65.0%)	10 (50%)	7 (46.7%)	10 (66.7%)	8 (61.5%)	82 (50.6%)
	Resistance	16 (39.0%)	29 76.3%)	7 (35.0%)	10 (50%)	8 (53.3%)	5 (33.3%)	5 (38.5%)	80 (49.4%)
Chloramphenicol	Sensitivity	32 (78.0%)	18 (47.4%)	13 (65.0%)	13 (65.0%)	10 (66.7%)	11 (73.3%)	9 (69.2%)	106 (65.4%)
	Resistance	9 (22.0%)	20 (52.6%)	7 (35.0%)	7 (35.0%)	5 (33.3%)	4 (26.7%)	4 (30.8%)	56 (34.6%)
Sparfloxacin	Sensitivity	35 (85.4%)	31 (81.6%)	17 (85.0%)	16 (80.0%)	15 (100.0%)	12 (80.0%)	10 (76.9%)	136 (84.0%)
	Resistance	6 (14.6%)	7 (18.4%)	3 (15.0%)	4 (20.0%)	0 (0.0%)	3 (20.0%)	3 (23.1%)	26 (16.0%)
Ciprofloxacin	Sensitivity	38 (92.7%)	32 (84.2%)	19 (95.0%)	17 (85.0%)	15 (100.0%)	14 (93.3%)	12 (92.3%)	147 (90.7%)
	Resistance	3 (7.3%)	6 (15.8%)	1 (5.0%)	3 (15.0%)	0 (0.0%)	1 (6.7%)	1 (7.7%)	15 (9.3%)
Gentamycin	Sensitivity	29 (70.7%)	21 (55.3%)	15 (75.0%)	11 (55.0%)	7 (46.7%)	11 (73.3%)	9 (69.2%)	103 (63.6%)
	Resistance	12 (29.3%)	17 (44.7%)	5 (25.0%)	9 (45.0%)	8 (53.3%)	4 (26.7%)	4 (30.8%)	59 (36.4%)
Perfloxacin	Sensitivity	37 (90.2%)	31 (81.6%)	15 (75.0%)	16 (80.0%)	13 (86.7%)	12 (80.0%)	10 (76.9%)	134 (82.7%)
	Resistance	4 (9.8%)	7 (18.4%)	5 (25.0%)	4 (20.0%)	2 (13.3%)	3 (20.0%)	3 (23.1%)	28 (17.3%)
Ofloxacin	Sensitivity	33 (80.5%)	26 (68.4%)	15 (75.0%)	16 (80.0%)	15 (100.0%)	12 (80.0%)	10 (76.9%)	127 (78.4%)
	Resistance	8 (19.5%)	12 (31.6%)	5 (25.0%)	4 (20.0%)	0 (0.0%)	3 (20.0%)	3 (23.1%)	35 (21.6%)
Streptomycin	Sensitivity	27 (65.9%)	20 (52.6%)	16 (80.0%)	11 (55%)	9 (60.0%)	10 (66.7%)	8 (61.5%)	101 (62.3%)
	Resistance	14 (34.1%)	18 (47.4%)	4 (20.0%)	9 (45%)	6 (40.0%)	5 (33.3%)	5 (38.5%)	61 (37.7%)

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Table 5: Antibiotic resistance patterns for Gram negative isolates

Antibiotics	**Amount (µg)**	**Sensitive**	**Resistant**
Ciprofloxacin	5	147 (90.7%)	15 (9.3%)
Sparfloxacin	10	136 (84.0%)	26 (16.0%)
Perfloxacin	10	134 (82.7%)	28 (17.3%)
Ofloxacin	5	127 (78.4%)	35 (21.6%)
Chloramphenicol	30	106 (65.4%)	56 (34.6%)
Gentamycin	10	103 (63.6%)	59 (36.4%)
Streptomycin	10	101 (62.3%)	61 (37.7%)
Septrin	30	82 (50.6%)	80 (49.4%)
Amoxicillin	30	66 (40.7%)	96 (59.3%)
Augmentin	30	61 (37.7%)	101 (62.3%)

Chart 3: Antibiotic resistance patterns for Gram negative isolates

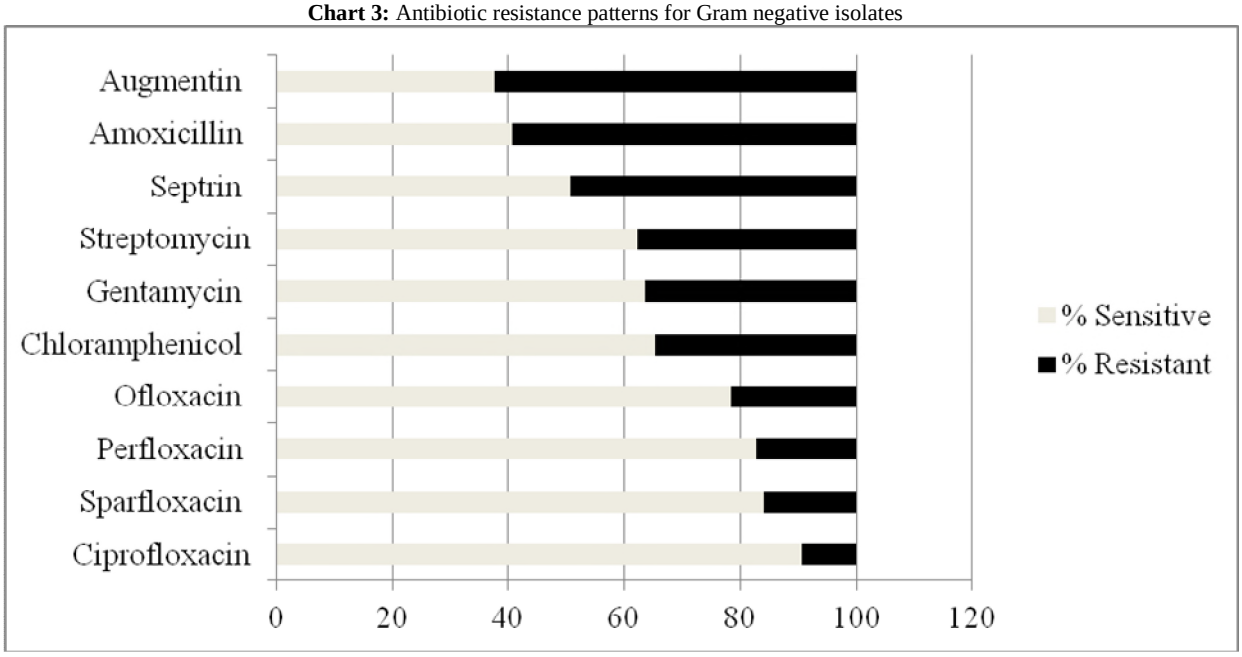

Table 6: Resistance patterns of Gram positive bacteria isolates.

Resistant Pattern	*Staphylococcus aureus*	Coagulase Negative *Staphylococcus species*	*Streptococcus species*
AM	1	0	0
APX,AM	1	1	1
APX, AM, Z	6	1	0
APX,AM, CN	0	1	0
APX, AM, Z,R	2	1	0
APX, Z, CN,S	1	0	0
APX,AM, CN,R	0	1	0
APX, AM, Z, CN,	1	0	1
APX, AM, Z,SXT	3	0	0
APX, AM, Z, PEF	0	0	1
APX, AM, Z, CN,R	3	0	0
APX, AM, Z,SXT,E	0	1	0
APX, AM, Z, CN, SXT,S	0	1	0
APX, AM, Z, CN, SXT, S,E	1	0	0
APX, AM, Z, CN,R,SXT,S	0	0	2
APX, AM, Z, CN, R, SXT, S,E	1	0	2

LEGEND: PEF- Perfloxacin, E- Erythromycin, SXT- Streptomycin, S- Septrin, CN- Gentamycin, Z- Zinnacef, APX- Ampiclox, AM- Amoxacillin

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Table 7: Resistance patterns of Gram negative bacteria isolates

Resistant pattern	*E. coli*	*Proteus species*	*Klebsiella species*	*Acinetobacter species*	*Pseudomonas species*	*Actinobacter species*	*Citrobacter species*
AU	0	0	1	0	2	2	1
SXT	1	1	0	0	0	0	0
GN	1	0	0	0	0	0	0
CH	0	0	1	0	0	0	1
CPX	0	0	0	0	0	0	1
AM	1	0	0	0	0	0	0
S	0	1	0	1	0	0	0
AU, SXT	0	2	0	0	0	0	0
AU, AM	2	0	0	0	2	1	0
AU, GN	0	0	0	1	0	0	0
SXT, CH	0	0	1	0	0	0	0
SXT, S	1	0	0	0	0	0	0
S, OFX	1	0	0	0	0	0	0
AU, S	0	0	0	0	1	0	0
AU, AM, PEF	0	0	0	0	0	1	0
AU , AM, CH	0	1	0	0	0	1	0
SXT, OFX, S	0	0	0	1	0	0	0
AU, AM, SXT	2	1	0	1	2	0	0
AU, GN, CH	0	1	0	0	0	0	0
AU, AM, S	0	1	0	0	0	0	0
AU, AM, GN	2	0	1	0	0	0	0
AU, SXT, CH,	0	0	2	1	0	0	0
AM, SXT, CH	0	1	0	0	0	0	0
S, AM, GN	0	0	0	1	0	0	0
AU, AM, GN, S	0	0	0	1	1	0	0
AU, AM, CH, SP	1	0	0	0	0	0	0
AU, AM, GN	1	1	0	0	1	0	0
AU, SXT, S, CH	0	0	0	0	0	0	1
AU, AM, GN, OFX	0	0	0	0	0	0	1
AU, AM, SXT, CH	0	1	0	1	0	0	0
AM, SXT, OFX, PEF	0	0	1	0	0	0	0
SXT, GN, CH, OFX	0	1	0	0	0	0	0
AU, AM, SXT, S	1	2	0	0	0	0	0
AU, SXT, GN, CH	0	0	0	1	1	0	0

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AU, AM, SXT, GN,CH	0	1	0	0	1	1	0
AU, AM, SXT, GN, S	1	1	0	0	1	0	0
AU, AM, SXT, S, CH	0	1	0	0	0	1	0
AU, AM, GN, OFX, PEF	0	1	0	0	0	0	1
AU, AM, SXT, S, OFX	1	0	0	0	0	0	0
AU, AM, SXT, CH, SP	0	1	0	0	0	0	0
AU, S, SXT, CH, OFX,	0	1	0	0	0	0	0
AU, AM, SXT, GN, S,	0	0	0	0	1	0	0
AU, AM, SXT, GN, CH	0	1	0	0	0	0	0
AM, SXT, S , CH, CPX	0	1	0	0	0	0	0
AU, AM, SXT, S, SP	1	0	0	0	0	0	1
AU, AM, GN, S, OFX, PEF	0	0	1	0	0	0	0
AU, AM, SXT, S, OFX, SP	0	1	0	0	0	0	0
AU, AM, SXT, GN, S, CH,	2	0	0	0	1	0	0
AU, AM, SXT, GN, S, OFX	0	0	0	0	0	0	1
AU, AM, GN, S, PEF, SP	0	0	0	1	0	0	0
AU, AM, SXT, S, CH, OFX	1	0	0	0	0	0	0
AU, AM, SXT, GN, S, CH	0	0	0	0	0	0	3
AU, AM, SXT, GN, S, OFX	0	0	0	0	0	1	0
AU, AM, SXT, GN, CH, OFX, PEF	0	1	0	0	0	0	0
AU, AM, SXT, GN, S, CH, OFX, PEF	1	0	0	0	0	0	0
AU, AM, SXT, GN, S, CH, OFX, PEF	0	0	1	0	0	0	0
AU, AM, SXT, GN, S, CH, OFX, CPX	0	0	1	0	0	0	0
AU, AM, SXT, GN, S, CH, OFX, SP, CPX	2	0	0	0	0	0	0
AU, AM, SXT, GN, S, CH, OFX, PEF, CPX	0	1	0	0	0	0	0
AU, AM, SXT, GN, S, CH, OFX, PEF, SP	1	0	2	0	0	0	1
AU, AM, SXT, GN, S, CH, OFX, PEF, SP, CPX	1	4	1	2	1	1	1

LEGEND:
AU- augmentin

(30µg), **GN**- gentamycin (10µg), **CPX**- ciprofloxacin (5µg), **CH**- chloramphenicol (30µg)
PEF- perfloxacin (10µg), **SXT**- streptomycin (10µg), **AM**- amoxicillin (30µg), **SP**- Sparfloxacin (10µg)
OFX- ofloxacin (5µg), **S** septrin (30µg)

Discussion and Conclusion

A total of 222 bacteria isolates were cultured from the 173 nasal swab samples, out of which 10 potentially pathogenic bacteria including 3 Gram positive bacteria (*Staphylococcus aureus* 14.0%, coagulase negative *Staphylococcus species* 7.7% and *Streptococcus species* 5.4%) and 7 Gram negative bacteria (*Escherichia coli* 18.5%, *Proteus species* 17.1%, *Klebsiella species* 9.0%, *Acinetobacter species* 9.0%, *Pseudomonas species* 6.8%, *Actinobacter species* 6.8% and *Citrobacter species* 5.9%) were identified. The most frequently isolated bacteria in decreasing order included *Escherichia coli* (18.5%), *Proteus species* (17.1%), *Staphylococcus aureus* (14.0%), *Klebsiella species* (9.0%), *Acinetobacter species* (9.0%), coagulase negative *Staphylococcus species* (7.7%), *Pseudomonas species* (6.8%), *Actinobacter species* (6.8%) and *Citrobacter species* (5.9%) (Table 1). This result is in close agreement with Bauer *et al.* 2003 who reported the presence of 17 different bacterial species of Enterobacteriaceae, *Staphylococci species* and *Streptococci species*, from nasal swabs of healthy dog. Most of these bacteria have been implicated in respiratory disease in dogs and human (Adaszek *et al.,* 2009; Meyer *et al.,* 2010; Lansing *et al.,* 2002; Spaterna *et al.,* 2012). The susceptible dogs may present varying clinical signs such as coughing, nasal discharge, sneezing, difficulty in breathing, fever, loss of appetite and lethargic behavior (Ayodhya *et al.,* 2013). In this study, *Bacillus species* was disregarded because this might have been obtained from the surrounding soil, though it can also cause respiratory disease (Maden *et al.,* 2000; Amin *et al.,* 2015).

Less than 50% of Gram positive isolates (31.7%) were sensitive to second generation cephalosporin- cefuroxine (zinnacef). The next drugs include Ampiclox (18.3% for Gram positive and 40.7% for Gram negative isolates), Amoxicillin (15.0% for Gram positive) and Augmentin (37.7% for Gram negative isolates). Owing to these low sensitivities, the selection of Amoxicillin and Ampiclox among drugs of choice for first line therapy of dog with pneumonia is weakened (Lesley, 2010) since anyone of these isolates can cause respiratory disease. The susceptibility level was highest to ciprofloxacin for Gram positive 58 (96.7%) and Gram negative (90.7%) bacteria (table 3 and 5). Other fluoroquinolones such as Perfloxacin, Sparfloxacin and Ofloxacin had sensitivity ranging from 78.4% to 93.3% (Tables 3 and 5).

A pathogen is multidrug resistant (MDR) when it is resistant to three or more antibiotics at any given time (Jan *et al*., 2004). The antibiotic susceptibility pattern suggested that the isolated bacteria have strains which possessed varying MDR genes. This finding was also reported by Davis *et al.,* 2014 who reported the presence of multiple antibiotic resistances of *Staphylococcus species* isolated from healthy dog and cats. However, *Staphylococcus aureus* strains were 100% sensitive to Ciprofloxacin while coagulase negative *Staphylococcus species* strains were 100% sensitive to Ciprofloxacin, Perfloxacin, Streptomycin and Erythromycin (Table 2). MDR among normal flora of clinically healthy dogs calls for a great attention since most of them had no record of antibiotic therapy. The growing antibiotic resistance trend among bacteria in humans and animals in both diseased and clinically healthy state, instigates a need for continuous research to avert the impending danger of antibiotic resistance (CDC, 2010; Coates *et al.,* 2002; FDA, 2000).

Interestingly, more than 80 percent of human contacts (82%) have had less than 50 cm face-to-face closeness with these dogs; thus possessing a great risk of acquiring MDR pathogens. These human contacts have high chance of inhaling aerosolized bacteria from the body surfaces such as skin, oral cavity, nasal cavity etc. (Henning *et al.,* 2001) especially when those areas are disturbed by hand rubbing on the fur, excessive exercise etc. If this contaminated air is breathed in by man, within three feet (90 cm), there is risk of acquiring such pathogen (W.H.O., 2005). The presence of MDR bacteria among healthy dogs suggested that these dogs (companion animals) are reservoir of multidrug-resistant potentially pathogenic bacteria, which may be transferred to human, especially the closest human contacts and others who handle them in unabated endangering habit. Such carriage poses an underlying risk of infection and this should be considered during handling of healthy dogs by all in-contact humans such as dog owners, veterinary personnel and students etc. Although, the settling of these airborne bacteria from these dogs on the human respiratory epithelium may not result into infection or disease state, the exchange of antibiotic resistant genes (plasmid) should not be taken lightly (Lee, 2003). Transfer of antibiotic resistant plasmid in bacteria has been documented (Lansing *et al.,* 2002). These can make an antibiotic susceptible bacterium to be resistant to same after acquiring resistant plasmid. It is of importance to implement strategies to reduce the rate of appearance and spread of resistance bacteria to allow new drug discovery to catch up with bacteria resistance development.

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