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 chased, can be pathogenic. Certain similarities between humans and dogs portends dangerous behavioral habits that could lead to zoonotic disease transmission. This study aimed to investigate human behavioral habits.

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%), perfloroxacin (17.3%), ofloxacin (21.6%), chloramphenicol (34.6%), gentamicin (36.4%), streptomycin (37.2%), septrafin (49.4%), amoxicillin (59.3%), augmentin (62.3%) while the Gram positive bacteria showed resistance to ciprofloxacin (3.3%), perfloroxacin (6.7%), erythromycin (13.3%), streptomycin (21.7%), rocephin (28.3%), gentamicin (36.7%), tianacel (68.3%), ampiclox (81.7%) and amoxicillin (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.

Keywords: 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 (Orodu et al., 2010). The relationship between dogs and people is so close, to a point of being almost inseparable, that dogs are involved in on average 100,000 deadly injuries annually in the US (Lee, 2003) - some being fatal. 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 (Orodu 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.
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 Methyline 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).

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 Methyline 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), perfloroxacin (10μg), zinnacef (30μg), gentamicin (10μg) and ciprofloxacin (5μg), perfloroxacin (10μg), streptomycin (10μg), amoxicillin (30μg), chloramphenicol (30μg)), septrin (30μg), sparfloroxacin (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%), 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, Seprtin, Streptomycin, Rocephin, Erythromycin, Perfloroxacin and Ciprofloxacin respectively. Streptococcus species (n=12) showed 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, Seprtin, Streptomycin, Erythromycin, Perfloroxacin 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, Seprtin, Streptomycin, Perfloroxacin, 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%) and 14 (34.1%), 4 (9.8%) and 3 (7.3%) to Amoxicillin, Augmentin, Seprtin, Streptomycin, Gentamycin, Chloramphenicol, Ofloxacin, Sparfloroxacin, Perfloroxacin 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 Amoxicillin, Augmentin, Seprtin, Chloramphenicol, Streptomycin, Gentamycin, Ofloxacin, Sparfloroxacin, Perfloroxacin and Ciprofloxacin respectively. Klebsiella species showed 7 (35.0%), 7 (35.0%), 6 (30.0%), 6 (30.0%), 5 (25.0%), 5 (25.0%), 4 (20.0%), 3 (15.0%) and 1 (5.0%) resistance to Seprtin, Chloramphenicol, Amoxicillin, Augmentin, Gentamycin, Ofloxacin, Sparfloroxcin, Perfloroxacin 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 Amoxicillin, Augmentin, Seprtin, Gentamycin, Streptomycin, Chloramphenicol, Ofloxacin, Sparfloroxcin, Perfloroxacin 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, Amoxicillin, Seprtin, Gentamycin, Streptomycin, Chloramphenicol, Perfloroxacin, Sparfloroxcin, 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, Amoxicillin, Seprtin, Streptomycin, Chloramphenicol,
Gentamicin, 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, Amoxicillin, Septrin, Chloramphenicol, Gentamicin, 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**      |               |
| Staphylococcus aureus           | 31 (14.0%)    |
| Coagulase negative Staphylococcus species | 17 (7.7%)    |
| Streptococcus species           | 12 (5.4%)     |
| **Gram negative isolates**      |               |
| 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
**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  | 8(25.0%)                  | 6(35.0%)                                 | 6(50.0%)                | 17(28.3%) |
| Gentamycin | Sensitivity | 24(74.2%)                 | 3(17.6%)                                 | 4(28.3%)                | 47(78.3%) |
|            | Resistance  | 7(22.6%)                  | 0(0.0%)                                  | 6(50.0%)                | 13(21.7%) |
| Septrin    | Sensitivity | 23(74.2%)                 | 4(28.3%)                                 | 10(83.3%)               | 56(93.3%) |
|            | Resistance  | 11(35.5%)                 | 6(35.0%)                                 | 2(16.7%)                | 1(1.7%)   |
| Streptomycin| Sensitivity| 24(74.2%)                | 25(71.7%)                                | 17(38.3%)               | 52(86.7%) |
|            | Resistance  | 11(35.5%)                 | 17(50.0%)                                | 2(16.7%)                | 2(3.3%)   |
| Perflloxacin| 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%)   |
| Perflloxacin   | 10          | 56(93.3%) | 4(6.7%)   |
| Erythromycin   | 10          | 52(86.7%) | 8(13.3%)  |
| Streptomycin   | 30          | 43(78.3%) | 13(21.7%) |
| Rocephin       | 25          | 38(71.7%) | 17(28.3%) |
| Septrin        | 30          | 38(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%) |
Chart 2: Antibiotic resistance patterns for Gram positive isolates
### 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 |
|---------------|-----------------|------------------------|-----------------------|--------------------------|-----------------------------|--------------------------|---------------------------|---------------------------|---------------|
| Amoxicillin   | 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%)               | 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%)    |
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- Perflloxacin, E- Erythromycin, SXT- Streptomycin, S- Septrin, CN- Gentamycin, Z- Zinnacef, APX- Ampicloxx, AM- Amoxicillin
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                 | 0                     | 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                   |
| 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 | 0 | 0 | 0 |
| AU, AM, SXT, GN, S, OFX | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| AU, AM, GN, S, PEF, SP | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| AU, AM, SXT, S, CH, OFX | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| AU, AM, GN, S, OFX | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| AU, AM, SXT, GN, S, CH, OFX, PEF | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| AU, AM, SXT, GN, S, CH, OFX, OFX, PEF | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| AU, AM, SXT, GN, S, CH, OFX, OFX, OFX, PEF | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| AU, AM, SXT, GN, S, CH, OFX, OFX, CPX | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| AU, AM, SXT, GN, S, CH, OFX, SP, CPX | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| AU, AM, SXT, GN, S, CH, OFX, SP, CPX, OFX, PEF, CPX | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| AU, AM, SXT, GN, S, CH, OFX, PEF, SP | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| AU, AM, SXT, GN, S, CH, OFX, PEF, SP, CPX | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| AU, AM, SXT, GN, S, CH, OFX, PEF, SP, CPX | 1 | 0 | 0 | 0 | 0 | 0 | 0 |

**LEGEND:**

AU- augmentin
GN- gentamycin (10μg)
CPX- ciprofloxacin (5μg)
CH- chloramphenicol (30μg)
PEF- perflaxin (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; Lansig 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- cefuroxime (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 Perflloxacin, Sparflloxacin and Oflloxacin 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, Perflloxacin, Streptomyycin 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 (Lansig 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.

Competing interest: The authors affirm that this study and its interpretations were not under any financial or otherwise competing interest.

Acknowledgements: The authors appreciate the technical and moral contributions of Mrs O.O. Orioke and Mrs A. Iyanda of the Department of Veterinary Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria.

References

1. Adaszek, L., Gorna, M., Zietek, J., Katrzuba, J. and Winiarczyk, S. (2009). Bacterial nosocomial infections in dogs and cats. Zyczie Weterynaryjne. 84: 805-808.
2. Ayodhya, S., Tirumala Rao, D.S., Narisima Reddy, Y., Syam Sundar, N. and Girish Kumar. V. (2013). Isolation and characterization of bacteria from canine respiratory diseases in and around Hyderabad city, Andhra Pradesh, India, Vet World. 6(9): 601-604, doi: 10.5455/vetworld.2013.601-604
3. Amin, M.; Rakhisi Z. and Ahmady A.Z. (2015). Isolation and Identification of Bacillus species from soil and evaluation of their antibacterial properties. Avicenna Journal of Clinical Microbiology and Infection 2(1): 23233
4. Barrow, G.I. and Feitham, R.K.A. (2003). Cowan and Steel’s manual for the identification of medical bacteria 3rd edition. Cambridge University Press Pp 158 & 226.
5. Bauer, N., Moritz, A. and Weiss, R. (2003). Comparison of bacterial growth in the upper and lower respiratory tract of healthy dogs. Tierarztliche Praxis. Ausga K, Kleintiere/ Heimtiere. 31: 92-98.
6. Bradshaw John, (2012). In defense of Dogs: Why dogs need our understanding. Penguin Books Ltd. ISBN-10: 014104649X.
7. CDC (2010). Get smart: know when antibiotics work. Centers for Disease Control, Atlanta, GA. www.cdc.gov/Features/GetSmart
8. Cherry, B., Burns, A., Johnson, G.S. (2004). Salmonella Typhimurium outbreak associated with veterinary clinic. Emerg Infect Dis. 10:2249–2251.
9. Meyer, C., Gisborne, V. and Rawdon, T. (2010). Respiratory disease outbreak in East Coast working dogs. MAF Investigation and Diagnostic Centre, Upper Hutt; Sandy McLachlan, New Zealand. Veterinary Pathology, Hamilton.
10. Coates, A., Hu, Y., Bax, R., Page, C. (2002). The future challenges facing the development of new antimicrobial drugs. Nat Rev Drug Discov. 1(11):895-910.
11. Craig E. Greene (2011). Infectious Diseases of the Dog and Cat, Fourth Edition. ISBN-13: 978-1416061304 ISBN-10: 1416061304.
12. Davis, J.A., Jackson, C.R., Fedorka-Cray, P.J., Barrett, J.B., Brousse, J.H., Gustafson, J. and Kucher, M. (2014). Carriage of methicillin-resistant staphylococci by healthy companion animals in the US. Letters in Applied Microbiology ISSN 0266- 8254. doi:10.1111/lam.12254
13. FDA (2000). FDA Task Force on Antimicrobial Resistance: key recommendations and report, Washington, DC. FDA. Washington, DC. http://www.fda.gov/downloads/ForConsumers/ConsumerUpdates/UCM143458.pdf
14. Gralton, J., Tovey, E., McLaws, M. L., and Rawlinson, W. D. (2011). The role of particle size in aerosolised pathogen transmission: a review. Journal of Infection 62(1): 1–13.
15. Health Canada (2005). An international outbreak of human salmonellosis associated with animal-derived pet treats — Canada and Washington state. Can Commun Dis Rep. 2006; 32:150–155.
16. Henning, S. and Marianne, S. (2001). Resistance to antibiotics in the normal flora of animals. INRA, EDP Sciences.Vet. Res. 32: 227–241.
17. Jan, M. B. John, D. T. and Sentry, P.A.C. (2004). High prevalence of oxacillin resistant Staphylococcus aureus isolates from hospitalized patients in Asia pacific and South Africa: Results from SENTRY antimicrobial surveillance program, 1998-1999. Antimicrob. Agent Chemother. 46: 879-881.
18. Lansing M. Prescott (2002). Microbiology 5th Edition. The McGraw−Hill Companies, pp 899-940
19. Lee, J. H. (2003). Methicillin (oxacillin)-resistant Staphylococcus aureus strains isolated from major food animals and their potential transmission to humans. Appl. Environ. Microbiol. 69:6489
20. Lesley G. King (2010).Treating canine bacterial pneumonia: more than just antibiotics. Proceedings of the International SCIVAC Congress 2010 - Rimini, Italy. pp 133-134
21. Maden, M., Birdane, M. F., Alkan, F., Hadimli, H. H. and Aslan, V. (2000) Clinical, cytologic, bacteriologic and radiographic analysis of respiratory diseases in dogs. Veteriner Bilimleri Dergisi. 16: 43-50.
22. Manian, F.A. (2003). Asymptomatic nasal carriage of mupirocin-resistant, methicillin-resistant Staphylococcus aureus (MRSA) in a pet dog associated with MRSA infection in household contacts. Clin Infect Dis.; 36:26–28.
23. Omudu, E.A, Okpe, G. and Adelusi, S.M. (2010). Study on Dog population in Makurdi, Nigeria (II): A survey of Ectoparasite infestation and its public health implications. Journal of research in forestry, wildlife and environment, Volume 2( 3); 85-93.
24. Spaterna, A. and Cuteri, V. (2012). Association between enrofloxacin and N-acetylcyesteine in recurrent bronchopneumopathies in dogs caused by biofilm producer bacteria. Journal of Animal and Veterinary Advances. 11: 462-469.
25. Udell M.A.R, and Wynne, C.D.L (2008).A review of Domestic Dogs’ (Canis familiaris) Human-like behaviours: or Why Behaviour Analyst should stop worrying and love their dogs. J. Exp Anal Behav. 89(2): 247-261.
26. Weese, J.S (2007). Infectious diseases of Dog and cat, 3rd ed. Can Vet. J. 48(1):75. PMCID: PMC1716741.
27. World Health Organization (2005). Practical Guidelines for Infection Control in Health Care Facilities. World Health Organization, Regional Office for Western Pacific, Manilla, Philippines Vol. 41 of SEARO Regional Publication.