**CHAPTER – I**

**INTRODUTCTION**

The relationship between people and dogs is unique. Among domesticated, 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 started 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. However, it is not entirely without any health risk. A high zoonotic risk is involved with the increasing number of people, as the people keep dogs for various purposes without much knowledge on the zoonoses (Omudu *et al.*, 2010). Dogs in Bangladesh often live in close proximity to humans and act as pathogen carriage, serve as a potential source of infection to humans ([Bruce and Fleming, 1983](https://scialert.net/fulltextmobile/?doi=ajava.2012.434.440#886285_ja); [Goossen *et al*., 1991](https://scialert.net/fulltextmobile/?doi=ajava.2012.434.440#885981_ja); [Burnens *et al* ., 1992](https://scialert.net/fulltextmobile/?doi=ajava.2012.434.440#862162_ja); [Ene *et al*., 1992](https://scialert.net/fulltextmobile/?doi=ajava.2012.434.440#886172_ja); [Moreno *et al*., 1993](https://scialert.net/fulltextmobile/?doi=ajava.2012.434.440#886094_ja); [Torre and Tello, 1993](https://scialert.net/fulltextmobile/?doi=ajava.2012.434.440#862332_ja); [Fernandez *et al*., 1994](https://scialert.net/fulltextmobile/?doi=ajava.2012.434.440#885958_ja); [Robinson and Pugh, 2002](https://scialert.net/fulltextmobile/?doi=ajava.2012.434.440#886139_ja); [Workman *et al*., 2005](https://scialert.net/fulltextmobile/?doi=ajava.2012.434.440#255295_ja); [Sabry, 2009](https://scialert.net/fulltextmobile/?doi=ajava.2012.434.440#834584_ja)). Thus the possibility of direct transmission of pathogens to human is more. Pathogenic organisms have long been recognized as a significant problem owing to their pathogenicity potential to animals and their zoonotic risk to humans. Among them many pathogens causing serious impact on public health ([Goldberg and Rubin, 1988](https://scialert.net/fulltextmobile/?doi=ajava.2012.434.440#885971_ja); [Baserisalehi *et al*., 2006](https://scialert.net/fulltextmobile/?doi=ajava.2012.434.440#124554_ja); [Humphrey *et al*., 2007](https://scialert.net/fulltextmobile/?doi=ajava.2012.434.440#358014_ja); [Ethelberg*et et al*., 2004](https://scialert.net/fulltextmobile/?doi=ajava.2012.434.440#885940_ja)). Importantly, frequent use of antibiotic against pathogens creating resistant microorganism and thus pathogens can acquire resistant factor which is transferable to human. 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).

Antimicrobial resistance is common problem all over the world. Antibiotic resistance in clinically healthy animals is continuously increasing too (Coates *et al.,* 2002; Davis *et al.,* 2014; Henning *et al*., 2001; Manian, 2003). However, there is a dearth of information and research on antimicrobial resistance in dogs in Bangladesh. Antibiotic resistance among many potential pathogenic organisms’ posses a great threat to human in this areas. Moreover, pathogens of the normal flora can easily acquire and transfer resistance genes. Thus regular monitoring of the level of resistance in pathogens of the normal flora has been recommended (Martel J-L *et al*., 2001).

Organisms of the nasal cavities and rectum of clinically healthy dogs may be zoonotic and may escape into the environment while the dog breaths and defecate. The behavioral habits of dog owners/lovers may expose them to pathogenic agents through inhalation, ingestion, skin contact etc. The transmission of disease can occur through both “droplet” and “feces”.

Therefore, this study was carried out to determine the status of antibiotic resistance of pathogenic organisms in nasal and rectum of apparently healthy dogs in order to provide updated information and the suspected role of dogs in its zoonotic significance.

**CHAPTER – II**

**MATERIALS & METHODS**

**2.1. Collection of samples**

Samples of nasal and rectal swabs were collected from the 10 dogs from SAQTVH, CVASU using sterile cotton swabs from March, 2018 to May, 2018 and transferred to the PRTC laboratory for culture and susceptibility testing. These dogs were used as guards, pets, and other various purposes in different areas of Chittagong. None of the dogs had apparent bacterial infections or was receiving antimicrobial therapy at the time of sample collection. Two samples one from nasal cavity and another from rectum were collected per animal. (Fig.1, 2) Structured questionnaire and closest interaction with dog owner were used to get the necessary information. Samples were collected and transported directly to the laboratory of PRTC for further analysis.

**2.2. Antimicrobial Susceptibility Test**

Samples were inoculated into 5ml buffer peptone water in a falcon tube for enrichment of bacteria. (Fig.3). The solutions were incubated at 37°C for 24 h. After incubation, a loop full of the buffer peptone water was inoculated onto 5% sheep blood agar to enrich the growth of bacterial colony. After enough growth of bacterial colonies, one colony was taken in to a test tube containing 3 ml PBS saline and match with 0.5% Mac Ferland turbidity standard in just eyes level (Fig.4). After matching of turbidity (0.5%), 1 swab sticks from this colony containing PBS saline were spread into fresh Mueller Hinton agar (Fig.5) and 9 different antimicrobial discs were inoculated (Fig.6) and again incubated at 37°C for 18 to 24 hours to observe and measure the zone of inhibition (Fig.7, 8).

The susceptibility of identified samples to antimicrobial agents was determined by the standard Kirby-Bauer disk diffusion method according to the method describe previously (Bauer AW *et al*., 1996; Woods GL & Washington JA., 1995). Zones of growth inhibition were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

Susceptibility to the following antimicrobials were determined for different samples: amoxycillin (30μg), ampicillin (30μg), ciprofloxacin (30μg), cefotaxime (30μg), doxycycline (30μg), gentamycin (30μg), cefixime (30μg), penicillin G (30μg), sulphamethaxazole (30μg).

**CHAPTER – III**

**RESULTS**

A total of 20 samples were obtained from 10 nasal swabs (table 1) and 10 rectal (table 2) swabs of apparently healthy dogs. The results of antibiotic sensitivity test for the nine antimicrobial agents for different samples were identified. Our result showed that, out of 20 samples amoxycillin 17 (85%), ampicillin 19 (95%), cefotaxime 11 (55%), ciprofloxacin 5 (25%), doxycline 11 (55%) and gentamycine 7 (35%) were resistant. All samples were found to be 100% resistant to cefixime, penicillin G and sulphamethaxazole (table 3).

**Table 1: Results of antibiotic sensitivities test where S=Sensitive, R=Resistant, N=nasal sample**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample** | **AX** | **AM** | **CTX** | **CIP** | **PO** | **GN** | **CFM** | **P** | **SXT** |
| N-1 | R | R | R | 20mm | 10mm | 12mm | R | R | R |
| N-2 | R | R | R | 18mm | 14mm | 8mm | R | R | R |
| N-3 | R | R | R | 14mm | R | R | R | R | R |
| N-4 | 10mm | R | R | 16mm | 12mm | R | R | R | R |
| N-5 | 8mm | R | 12mm | R | 10mm | 18mm | R | R | R |
| N-6 | R | R | R | R | R | R | R | R | R |
| N-7 | 16mm | 10mm | 14mm | 20mm | R | 10mm | R | R | R |
| N-8 | R | R | R | 16mm | R | 12mm | R | R | R |
| N-9 | R | R | 10mm | 14mm | R | 16mm | R | R | R |
| N-10 | R | R | 12mm | 20mm | 8mm | 14mm | R | R | R |

**LEGEND: GN-**Gentamycin (10μg), **CIP-**Ciprofloxacin (5μg), **AX-**Amoxicillin (30μg), **AM-**Ampicillin(30μg), **CTX-**Cefotaxime(30μg), **DO-**Doxycycline(30μg), **CFM-**Cefixime, **P-**Penicillin G(30μg), **SXT-**Sulphamethaxazole(30μg).

**Table 2: Results of antibiotic sensitivities test where S=Sensitive, R=Resistant, F=fecal sample**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample** | **AX** | **AM** | **CTX** | **CIP** | **PO** | **GN** | **CFM** | **P** | **SXT** |
| F-1 | R | R | 8mm | 18mm | 8mm | 16mm | R | R | R |
| F-2 | R | R | R | 14mm | R | 10mm | R | R | R |
| F-3 | R | R | R | R | R | 18mm | R | R | R |
| F-4 | R | R | 14mm | 10mm | 12mm | R | R | R | R |
| F-5 | R | R | R | R | R | R | R | R | R |
| F-6 | R | R | 10mm | 12mm | R | 8mm | R | R | R |
| F-7 | R | R | 12mm | 18mm | R | R | R | R | R |
| F-8 | R | R | R | R | 8mm | 10mm | R | R | R |
| F-9 | R | R | R | 14mm | R | 14mm | R | R | R |
| F-10 | R | R | 10mm | 12mm | 6mm | R | R | R | R |

**LEGEND: GN-**Gentamycin (10μg), **CIP-**Ciprofloxacin (5μg), **AX-**Amoxicillin (30μg), **AM-**Ampicillin(30μg), **CTX-**Cefotaxime(30μg), **DO-**Doxycycline(30μg), **CFM-**Cefixime, **P-**Penicillin G(30μg), **SXT-**Sulphamethaxazole(30μg).

**Table 3: Results of antibiotic sensitivities test of antimicrobial agents**

Number of total samples: 20

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Antimicrobial Agents** | **Sensitivity** | **Resistant** | **Sensitivity(%)** | **Resistant(%)** |
| Amoxycillin(AX) | 3 | 17 | 15% | 85% |
| Ampicillin(AM) | 1 | 19 | 5% | 95% |
| Cefotaxime(CTX) | 9 | 11 | 45% | 55% |
| Ciprofloxacine(CIP) | 15 | 5 | 75% | 25% |
| Doxycycline(DO) | 9 | 11 | 45% | 55% |
| Gentamycine(CN) | 13 | 7 | 65% | 35% |
| Cefixime(CFM) | 0 | 20 | 0% | 100% |
| Penicillin G(P) | 0 | 20 | 0% | 100% |
| Sulphamethaxazole(SXT) | 0 | 20 | 0% | 100% |

**CHAPTER – IV**

**Discussion and Conclusion**

Laboratory culture and susceptibility results are usually used for monitoring antimicrobial resistance. In this study, a total of 20 samples were cultured included 10 nasal and 10 rectal swabs to see the antimicrobial resistance pattern in dog brought to the SAQTVH, CVASU.

This study can only support the fact about development and prevailing antimicrobial resistance among bacteria. We found that, all isolates were sensitive to ciprofloxacine and gentamycine. It may be due to least use of these drugs in dog now days. Majority of the isolates were resistant to amoxycillin, ampicillin, cefixime, penicillinG and sulphamethaxazole. These are the drugs commonly using to treat the dog in this hospital. Rate of resistance in dogs and other animals may range from 15 to 94% ([Modolo *et al*., 1991](https://scialert.net/fulltextmobile/?doi=ajava.2012.434.440#886293_ja); [Gaudreau and Gilbert, 1998](https://scialert.net/fulltextmobile/?doi=ajava.2012.434.440#885966_ja); [Saenz *et al*., 2000](https://scialert.net/fulltextmobile/?doi=ajava.2012.434.440#269925_ja); [De Vega *et al*., 2005](https://scialert.net/fulltextmobile/?doi=ajava.2012.434.440#116045_ja)) which is supporting our findings. We also got this range of resistance in our findings. In the present work, the great variability in this antibiotic's efficacy is probably due to its worldwide use both at therapeutic or low doses. We found the gentamycin resistance though the gentamycin is not commonly using in dogs. Increased resistance of these antimicrobials was observed may be due to genetic mutations interfering with bacterial DNAgirase. Prudent use of antimicrobials is an important step in reducing the emergence of antimicrobial resistance. Pathogen randomly selected from the study population is susceptible to a particular antimicrobial on disk diffusion testing are likely to be susceptible to antimicrobials. Other factors including pharmacokinetics, antimicrobial use strategies to reduce the emergence of resistance, drug safety profile, cost and convenience of administration must be considered. However, almost all antimicrobials are consistent with increasing antimicrobial resistance. Antimicrobial resistance observed might be due to the indiscriminate and irrational use of antimicrobials ([Tambekar *et al*., 2007](https://scialert.net/fulltextmobile/?doi=ajava.2012.434.440#123363_ja)) in animals for preventive or therapeutic purposes irrespective of etiological agents.

It is of importance to implement strategies to reduce the spread of resistance otherwise new drug discovery also showed resistance development. Culture and susceptibility testing for individual cases remains the best instrument for guiding treatment decisions, especially for recurrent infections. Increasing antimicrobial resistance is a growing concern in both human and veterinary medicine. Because pathogens isolated from recurrent infections are more resistant and resistance is increasing over time, appropriate management of recurrent infections is critical to control antimicrobial resistance. A continuous surveillance and monitoring of the antimicrobial resistance in dogs and other pet animals is essential to the implementation of effective policies for controlling and preventing contamination and infection by this pathogen. The use of antibiotics as therapeutic and prophylaxis for animals should be carefully evaluated and monitored because acquisition of antibiotic resistant strains by man has serious health implications. 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). Underlying anatomic or metabolic problems should be identified and addressed whenever possible. In order to detect early changes in bacterial susceptibilities before a high resistance is selected or developed, regular monitoring of antimicrobial resistance in normal flora of companion animals will be needed. Since the number of reports from different drug resistance mechanisms in canine is scarce, more studies will be needed.

**PHOTO GALARRY**

Fig. 2: Collection of fecal swab

Fig.1: Collection of nasal swab

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Fig.4: Making of Standard dilution

Fig.3: Inoculation of sample into 5ml buffer peptone

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Fig.6: Inserting antimicrobial discs

Fig.5: Sticking sample into fresh Mueller Hinton agar

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Fig.8: Observation of result

Fig.7: Incubation at 370C

**CHAPTER-V**

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 **The Author**

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**BIOGRAPHY**

I’m **Joya Dhar Mumu**, an intern student at Chittagong Veterinary and Animal Sciences University (CVASU), originate from Chittagong. After completing one year intern period, I will receive my Doctor of Veterinary Medicine (DVM) degree with lots of real life experiences. As an intern student I’ve received clinical training from Madras Veterinary College and Veterinary College & Research Institute, Namakkal, Tamilnadu, India. I have more interest on theriogenology, medicine, surgery, microbiology and epidemiological field area.